Burke
09/126816

- key terms

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     FILE 'CAPLUS' ENTERED AT 11:32:29 ON 16 NOV 1999
L1         2453 S RAS(S)(P21 OR PROTO?)
L2            1 S L1 AND (TOXIN(S)LT OR LETHAL TOXIN)
L3           22 S RAS AND (TOXIN(S)LT OR LETHAL TOXIN)
L4           20 S L3 AND SORDELL?
L5           20 S L2 OR L4
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L5 ANSWER 1 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:583555 CAPLUS
DOCUMENT NUMBER: 131:209119
TITLE: Toxicologically active fragments of **lethal toxin** from Clostridium **sordellii** and their application in immunotoxins
INVENTOR(S): Aktories, Klaus; Hofmann, Fred
PATENT ASSIGNEE(S): Albert-Ludwigs-Universitaet Freiburg, Germany
SOURCE: Ger. Offen., 14 pp.
CODEN: GWXXBX
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19802569	A1	19990909	DE 1998-19802569	19980123

AB Fragment 1-546 of C. **sordellii lethal toxin** and an immunotoxin comprising this protein fused to a cell-binding moiety, such as a tumor cell-binding antibody or antibody fragment, are disclosed. The immunotoxin may addnl. contain a translocation signal, e.g., the translocation domain of Pseudomonas exotoxin A or of the Clostridium C2 toxin. The 1-546 fragment of the C. **sordellii lethal toxin** was found to have higher glucosyltransferase activity with **Ras** as substrate than did the wild-type **lethal toxin**.

L5 ANSWER 2 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:486949 CAPLUS
DOCUMENT NUMBER: 131:238263
TITLE: Rundown of somatodendritic N-methyl-D-aspartate (NMDA) receptor channels in rat hippocampal neurones: evidence for a role of the small GTPase RhoA
AUTHOR(S): Norenberg, Wolfgang; Hofmann, Fred; Illes, Peter; Aktories, Klaus; Meyer, Dieter K.
CORPORATE SOURCE: Department of Pharmacology, Albert-Ludwigs-University, Freiburg, D-79104, Germany
SOURCE: Br. J. Pharmacol. (1999), 127(5), 1060-1063

CODEN: BJPCBM; ISSN: 0007-1188
PUBLISHER: Stockton Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Actin filament (F-actin) depolymn. leads to the use-dependent rundown of N-methyl-D-aspartate (NMDA) receptor activity in rat hippocampal neurons. Depolymn. is promoted by Ca2+ which enters the cells via NMDA receptor channels. The **ras** homolog (Rho) GTPases (RhoA, Rac1 and Cdc42) promote actin polymn. and thus control the actin cytoskeleton. We have investigated, by means of the whole-cell patch clamp technique, whether the actin fibers which interact with NMDA receptors are controlled by Rho GTPases. In the presence of intracellular ATP which attenuates rundown, the C3 toxin from Clostridium (C.) botulinum was used to inactivate RhoA. Indeed, it enhanced the use-dependent rundown of NMDA-evoked inward currents to a level similar to that obtained in the absence of ATP. **Lethal toxin** from Clostridium **sordellii** which inactivates Rac1 and Cdc42 lacked this effect. We suggest that the function of somatodendritic NMDA receptor channels in rat hippocampal neurons can be modulated by RhoA via its action on F-actin.

L5 ANSWER 3 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:351907 CAPLUS
DOCUMENT NUMBER: 131:98722
TITLE: G-protein-stimulated phospholipase D activity is inhibited by **lethal toxin** from Clostridium **sordellii** in HL-60 cells
AUTHOR(S): El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud, Jean-Christophe; Payrastre, Bernard; Boquet, Patrice; Geny, Blandine
CORPORATE SOURCE: INSERM U332, ICGM, Paris, 75014, Fr.
SOURCE: J. Biol. Chem. (1999), 274(20), 14021-14031
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Lethal toxin** (**LT**) from Clostridium **sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 3-acetate via the protein kinase C pathway. The toxin effect on

actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease obsd. in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in Lt-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by **LT** and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as **LT toxin** (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prepd. from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase C.alpha. was decreased after LT treatment. We conclude that in HL-60 cells, **lethal toxin** from C. **sordellii**, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications obsd. in HL-60 cells.

L5 ANSWER 4 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:350413 CAPLUS
DOCUMENT NUMBER: 131:142850
TITLE: Effects of cytotoxic necrotizing factor 1 and **lethal toxin** on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers
AUTHOR(S): Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; Boquet, Patrice; Van Obberghen-Schilling, Ellen
CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, Nice, 06108, Fr.
Searcher : Shears 308-4994

SOURCE: Infect. Immun. (1999), 67(6), 3002-3008
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study, the authors have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activated Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induced prominent stress fiber formation without modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocked thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (Clostridium botulinum) eliminated actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. **Lethal toxin** (Clostridium **sordellii**), an inhibitor of Rac as well as **Ras** and Rap, potently disrupted the actin microfilament system and monolayer integrity in HUVEC cultures.

L5 ANSWER 5 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:208526 CAPLUS
DOCUMENT NUMBER: 131:2919

TITLE: **Ras** family proteins: new players involved in the diplotene arrest of Xenopus oocytes

AUTHOR(S): Jessus, Catherine; Rime, Helene; Ozon, Rene
CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction, Inra/CNRS ESA 7080, Universite Pierre-et-Marie-Curie, Paris, 75252, Fr.

SOURCE: Biol. Cell (1998), 90(8), 573-583
CODEN: BCELDF; ISSN: 0248-4900
PUBLISHER: Editions Scientifiques et Medicales Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with 78 refs. Oogonia undergo numerous mitotic cell cycles before completing the last DNA replication and entering the meiotic prophase I. After chromosome pairing and chromatid exchanges between paired chromosomes, the oocyte I remains arrested at the diplotene stage of the 1st meiotic prophase. Oocyte growth then occurs independently of cell division; indeed, during this

growth period, oocytes (4n DNA) are prevented from completing the meiotic divisions. How is the prophase arrest regulated. One of the players of the prophase block is the high level of intracellular cAMP, maintained by an active adenylate cyclase. By using **lethal toxin** from Clostridium **sordellii** (**LT**), a glucosyl-transferase that glucosylates and inactivates small G proteins of the **Ras** subfamily, we have shown that inhibition of either **Ras** or Rap or both proteins is sufficient to release the prophase block of Xenopus oocytes in a cAMP-dependent manner. The implications of **Ras** family proteins as new players involved in the prophase arrest of Xenopus oocytes will be discussed here.

L5 ANSWER 6 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:62268 CAPLUS
DOCUMENT NUMBER: 130:206160
TITLE: Inhibition of small G proteins by Clostridium **sordellii lethal toxin** activates cdc2 and MAP kinase in Xenopus oocytes
AUTHOR(S): Rime, Helene; Talbi, Nabila; Popoff, Michel R.; Suziedelis, Kestutis; Jessus, Catherine; Ozon, Rene
CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction, INRA/ESA-CNRS 7080, Universite Pierre et Marie Curie, Paris, 75252, Fr.
SOURCE: Dev. Biol. (1998), 204(2), 592-602
CODEN: DEBIAO; ISSN: 0012-1606
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **lethal toxin** (**LT**) from Clostridium **sordellii** is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. LT induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown Xenopus oocytes. Toxin B from Clostridium difficile, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras**-and(or) Rap, neg. regulate cdc2 kinase activation in Xenopus oocyte. In oocyte exts., LT catalyzes the incorporation of [14C]glucose into a group of proteins of 23 kDa and into 1 protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of LT into oocytes together with UDP-[14C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of LT. In vivo time-course anal. reveals that the 27-kDa protein glucosylation is completed within 2 h, well

before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of LT allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD. (c) 1998 Academic Press.

L5 ANSWER 7 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:691068 CAPLUS
DOCUMENT NUMBER: 130:122632
TITLE: Activation of a Ca2+-dependent K+ current in mouse fibroblasts by lysophosphatidic acid requires a pertussis toxin-sensitive G protein and **Ras**
AUTHOR(S): Repp, Holger; Koschinski, Andreas; Decker, Katrin; Dreyer, Florian
CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, Giessen, D-35392, Germany
SOURCE: Naunyn-Schmiedeberg's Arch. Pharmacol. (1998), 358(5), 509-517
CODEN: NSAPCC; ISSN: 0028-1298
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lysophosphatidic acid (LPA) is a bioactive lipid that acts through G protein-coupled plasma membrane receptors and mediates a wide range of cellular responses. Here, we report that LPA activates a K+ current in NIH3T3 mouse fibroblasts that leads to membrane hyperpolarization. The activation occurs with an EC50 value of 1.7 nM LPA. The K+ current is Ca2+-dependent, voltage-independent, and completely blocked by the K+ channel blockers charybdotoxin, margatoxin, and iberiotoxin with IC50 values of 1.7, 16, and 62 nM, resp. The underlying K+ channels possess a single channel conductance of 33 pS in sym. K+ soln. Pretreatment of cells with pertussis toxin (PTX), Clostridium **sordellii lethal toxin**, or a farnesyl protein transferase inhibitor reduced the K+ current amplitude in response to LPA to about 25% of the control value. Incubation of cells with the protein tyrosine kinase inhibitor genistein or microinjection of the neutralizing anti-**Ras** monoclonal antibody Y13-259 reduced it by more than 50%. In contrast, the phospholipase C inhibitor U-73122 and the protein kinase A activator 8-bromo-cAMP had no effect. These results indicate that the K+ channel activation by LPA is mediated by a signal transduction pathway involving a PTX-sensitive G protein, a protein tyrosine kinase, and **Ras**. LPA is already known to activate Cl- channels in various cell types, thereby leading to membrane depolarization. In conjunction

with our results that demonstrate LPA-induced membrane hyperpolarization by activation of K+ channels, LPA appears to be significantly involved in the regulation of the cellular membrane potential.

L5 ANSWER 8 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:524147 CAPLUS
DOCUMENT NUMBER: 129:227404
TITLE: A common motif of eukaryotic glycosyltransferases is essential for the enzyme activity of large clostridial cytotoxins
AUTHOR(S): Busch, Christian; Hofmann, Fred; Selzer, Jorg; Munro, Sean; Jeckel, Dieter; Aktories, Klaus
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Freiburg, D-79104, Germany
SOURCE: J. Biol. Chem. (1998), 273(31), 19566-19572
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A fragment of the N-terminal 546 amino acid residues of Clostridium **sordellii lethal toxin** possesses full enzyme activity and glucosylates Rho and **Ras** GTPases in vitro. Here we identified several amino acid residues in C. **sordellii lethal toxin** that are essential for the enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased glucosyltransferase activity by about 5000-fold and completely blocked glucohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of C. **sordellii lethal toxin** was labeled by azido-UDP-glucose after UV irradn., mutation of the DXD motif prevented radiolabeling. At high concns. (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced glucosyltransferase activity by about 200-fold and blocked glucohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large no. of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.

L5 ANSWER 9 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:425281 CAPLUS
DOCUMENT NUMBER: 129:158435

TITLE: Functional consequences of monoglucosylation of Ha-**Ras** at effector domain amino acid threonine 35

AUTHOR(S): Herrmann, Christian; Reza, Mohammad Reza; Hofmann, Fred; Just, Ingo

CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Frieburg, Hermann-Herder-Strasse, D-79104, Germany

SOURCE: J. Biol. Chem. (1998), 273(26), 16134-16139
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Monoglucosylation of low mol. mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. Ha-**Ras** is monoglucosylated at effector domain amino acid threonine 35 by Clostridium **sordellii lethal toxin**, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the mol. consequences of this modification, effects of glucosylation on each step of the GTPase cycle of **Ras** were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120GAP and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated **Ras**. Guanine nucleotide exchange factor (Cdc25)-catalyzed GTP loading was decreased, but not completely inhibited. A dominant-neg. property of modified **Ras** to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, **Ras**-effector coupling, was completely blocked. The Kd for the interaction between **Ras** -GTP and the **Ras**-binding domain of Raf was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of **Ras**.cntdot.GDP for Raf (Kd = 22 .mu.M) is too low to allow functional interaction, a glucose moiety at threonine 35 of **Ras** seems to block completely the interaction with Raf. The net effect of **lethal toxin**-catalyzed glucosylation of **Ras** is the complete blockade of **Ras** downstream signaling.

L5 ANSWER 10 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:271846 CAPLUS

DOCUMENT NUMBER: 129:52440

TITLE: Rho protein inhibition blocks protein kinase C translocation and activation

AUTHOR(S): Hippenstiel, Stefan; Kratz, Thomas; Krull, Matthias; Seybold, Joachim; Eichel-Streiber, Christoph V.; Suttorp, Norbert

CORPORATE SOURCE: Department of Internal Medicine,

Justus-Liebig-University, Giessen, D-35392, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1998), 245(3), 830-834
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium **sordellii lethal toxin**-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as detd. by PKC-activity measurements and Western blots for PKC.alpha.. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L5 ANSWER 11 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:223590 CAPLUS
DOCUMENT NUMBER: 128:318211

TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium **sordellii lethal toxin** and Clostridium difficile toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases

AUTHOR(S): Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; De Gunzburg, Jean; Von Eichel-Streiber, Christoph; Jakobs, Karl H.
CORPORATE SOURCE: Universitatsklinikum Essen, Institut fur Pharmakologie, Essen, D-45122, Germany

SOURCE: J. Biol. Chem. (1998), 273(13), 7413-7422
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To study whether **Ras**-like GTPases are involved in phospholipase D (PLD) regulation, we studied the effects of the Clostridium difficile toxin B (TcdB) variant TcdB-1470 and Clostridium **sordellii lethal toxin** (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by m3 muscarinic acetylcholine receptor (mAChR) or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time-and concn.-dependent manner, without alteration in immunol. detectable protein kinase C (PKC) isoenzyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addn. of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addn. of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addn. of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddn. of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L5 ANSWER 12 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:207001 CAPLUS
DOCUMENT NUMBER: 129:14892
TITLE: Activation of a Ca2+-dependent K+ current by the oncogenic receptor protein tyrosine kinase v-Fms in mouse fibroblasts
AUTHOR(S): Decker, Katrin; Koschinski, Andreas; Trouliaris, Sylvia; Tamura, T.; Dreyer, Florian; Repp, H.
CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, Giessen, D-35392, Germany
SOURCE: Naunyn-Schmiedeberg's Arch. Pharmacol. (1998), 357(4), 378-384
CODEN: NSAPCC; ISSN: 0028-1298
Searcher : Shears 308-4994

PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We investigated the effects of the receptor-coupled protein tyrosine kinase (RTK) v-Fms on the membrane current properties of NIH3T3 mouse fibroblasts. We found that v-Fms, the oncogenic variant of the macrophage colony-stimulating factor receptor c-Fms, activates a K+ current that is absent in control cells. The activation of the K+ current was Ca2+-dependent, voltage-independent, and was completely blocked by the K+ channel blockers charybdotoxin, margatoxin and iberiotoxin with IC50 values of 3 nM, 18 nM and 76 nM, resp. To identify signaling components that mediate the activation of this K+ current, NIH3T3 cells that express different mutants of the wildtype v-Fms receptor were examd. Mutation of the binding site for the **Ras**-GTPase-activating protein led to a complete abolishment of the K+ current. A redn. of 76% and 63%, resp., was obsd. upon mutation of either of the two binding sites for the growth factor receptor binding protein 2. Mutation of the ATP binding lobe, which disrupts the protein tyrosine kinase activity of v-Fms, led to a 55% redn. of the K+ current. Treatment of wild-type v-Fms cells with Clostridium **sordellii lethal toxin** or a farnesyl protein transferase inhibitor, both known to inhibit the biol. function of **Ras**, reduced the K+ current amplitude to 17% and 6% of the control value, resp. This is the first report showing that an oncogenic RTK can modulate K+ channel activity. Our results indicate that this effect is dependent on the binding of certain **Ras**-regulating proteins to the v-Fms receptor and is not abolished by disruption of its intrinsic protein tyrosine kinase activity. Furthermore, our data suggest that **Ras** plays a key role for K+ channel activation by the oncogenic RTK v-Fms.

L5 ANSWER 13 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:130788 CAPLUS
DOCUMENT NUMBER: 128:253966
TITLE: Chimeric clostridial cytotoxins: identification of the N-terminal region involved in protein substrate recognition
AUTHOR(S): Hofmann, Fred; Busch, Christian; Aktories, Klaus
CORPORATE SOURCE: Institute fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Freiburg, D-79104, Germany
SOURCE: Infect. Immun. (1998), 66(3), 1076-1081
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Clostridium **sordellii lethal toxin** is a member of the family of large clostridial cytotoxins that

glucosylate small GTPases. In contrast to Clostridium difficile toxins A and B, which exclusively modify Rho subfamily proteins, C. **sordellii lethal toxin** also glucosylates **Ras** subfamily proteins. By deletion anal. and construction of chimeric fusion proteins of C. **sordellii lethal toxin** and C. difficile toxin B, we localized the enzyme activity of the **lethal toxin** to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of C. **sordellii lethal toxin** glucosylated Rho and **Ras** subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of **lethal toxin** for those in the active toxin B fragment (1 to 546) allowed glucosylation of **Ras** subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from **lethal toxin**, and 469 to 546 from toxin B exhibited markedly reduced modification of **Ras** subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of C. **sordellii lethal toxin**.

L5 ANSWER 14 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1997:713569 CAPLUS
DOCUMENT NUMBER: 128:10495
TITLE: Evidence for differential roles of the Rho subfamily of GTP-binding proteins in glucose- and calcium-induced insulin secretion from pancreatic .beta. cells
AUTHOR(S): Kowluru, Anjaneyulu; Li, Guodong; Rabaglia, Mary E.; Segu, Venkatesh B.; Hofmann, Fred; Aktories, Klaus; Metz, Stewart A.
CORPORATE SOURCE: WILLIAM S. MIDDLETON MEMORIAL VA MEDICAL CENTER, MEDICAL AND RESEARCH SERVICES, MADISON, WI, 53705, USA
SOURCE: Biochem. Pharmacol. (1997), 54(10), 1097-1108
CODEN: BCPCA6; ISSN: 0006-2952
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We utilized clostridial toxins (with known specificities for inhibition of GTPases) to ascertain the contribution of candidate GTPases in physiol. insulin secretion from .beta. cells. Exposure of normal rat islets or isolated .beta. (HIT-T15) cells to Clostridium difficile toxins A and B catalyzed the glucosylation (and thereby the inactivation) of Rac, Cdc42, and Rho endogenous to

.beta. cells; concomitantly, either toxin reduced glucose- or potassium-induced insulin secretion from rat islets and HIT cells. Treatment of .beta. cells with Clostridium **sordellii lethal toxin** (**LT**; which modified only **Ras**, Rap, and Rac) also reduced glucose- or potassium-induced secretion. However, clostridial toxin C3-exoenzyme (which ADP-ribosylates and inactivates only Rho) was without any effect on either glucose- or potassium-induced insulin secretion. These data suggest that Cdc42, Rac, **Ras**, and/or Rap (but not Rho) may be needed for glucose- or potassium-mediated secretion. The effects of these **toxins** appear to be specific on stimulus-secretion coupling, since no difference in metabolic viability (assessed colorimetrically by quantitating the conversion of the tetrazolium salt into a formazan in a redn. reaction driven by nutrient metab.) was demonstrable between control and **toxin** (A or **LT**)-treated .beta. cells. **Toxin** (A or **LT**) treatment also did not alter glucose- or potassium-mediated rises in cytosolic free calcium concns. ([Ca2+]i), suggesting that these GTPases are involved in steps distal to elevations in [Ca2+]i. Recent findings indicate that the carboxyl methylation of Cdc42 is stimulated by only glucose, whereas that of Rap (A. Kowluru et al., 1996) and Rac (present study) are regulated by glucose or potassium. Together, these findings provide direct evidence, for the first time, that the Rho subfamily of GTPases plays a key regulatory role(s) in insulin secretion, and they suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas Rap and/or Rac may be required for a later step(s) in the stimulus-secretion coupling cascade (i.e. Ca2+-induced exocytosis of insulin).

L5 ANSWER 15 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:533546 CAPLUS
DOCUMENT NUMBER: 127:195467
TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor
INVENTOR(S): Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany; Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9727871 A1 19970807 WO 1997-EP426 19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9715982 A1 19970822 AU 1997-15982 19970131
EP 877622 A1 19981118 EP 1997-902278 19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
PRIORITY APPLN. INFO.: EP 1996-101469 19960202
WO 1997-EP426 19970131

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras proto**-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium **sordellii toxin LT** under conditions favoring inactivating of **Ras** by glucosylation of **Ras**' threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of **toxin LT**.

L5 ANSWER 16 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:510330 CAPLUS
DOCUMENT NUMBER: 127:172444
TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase
AUTHOR(S): Fiorentini, Carla; Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; Boquet, Patrice
CORPORATE SOURCE: Dep. Ultrastructures, Inst. Superiore Sanita, Rome, 00161, Italy
SOURCE: J. Biol. Chem. (1997), 272(31), 19532-19537
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of

Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent mol. wt. of the mol. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficult **toxin** B, which inactivates Rho but not those of Clostridium **sordellii LT toxin**, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-assocd. phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L5 ANSWER 17 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:761992 CAPLUS
DOCUMENT NUMBER: 126:43823
TITLE: Difference in protein substrate specificity between hemorrhagic toxin and **lethal toxin** from Clostridium **sordellii**
AUTHOR(S): Genth, Harald; Hofmann, Fred; Selzer, Joerg; Aktories, Klaus; Just, Ingo
CORPORATE SOURCE: Institut fuer Pharmakologie der Albert-Ludwigs-Universitaet Freiburg, Freiburg, D-79104, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1996), 229(2), 370-374
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Here we report that hemorrhagic toxin (HT), which is coexpressed with **lethal toxin**, is also a glucosyltransferase. Whereas **lethal toxin** glycosylates the Rho subfamily proteins Rac and Cdc42 and the **Ras** subfamily proteins H-**Ras** and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to **lethal toxin**, transferase activity of HT is stimulated by Mn2+. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. **sordellii** HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and **Ras** of GTPases.

L5 ANSWER 18 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:606610 CAPLUS
DOCUMENT NUMBER: 125:240634
TITLE: The **Ras**-related protein Ral is monoglucosylated by Clostridium **sordellii lethal toxin**
AUTHOR(S): Hofmann, Fred; Rex, Gundula; Aktories, Klaus; Just, Ingo
CORPORATE SOURCE: Institut fuer Pharmakologie und Toxikologie, AlbertLudwigs-Universitaet Freiburg, Freiburg, D-79104, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1996), 227(1), 77-81
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We report here on **lethal toxin** (**LT**) produced by C. **sordellii** strain 6018 which glucosylates in addn. to Rac, **Ras** and Rap the Ral protein. LT from strain VPI9048 however does not glucosylate Ral. Besides recombinant Ral, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equiv. to threonine-35 in H-**Ras** located in the effector region. The Ral-glucosylating toxin is a novel isoform of **Ras**-modifying clostridial cytotoxins.

L5 ANSWER 19 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1996:256012 CAPLUS
DOCUMENT NUMBER: 124:309937
TITLE: **Ras**, Rap, and Rac small GTP-binding proteins are targets for Clostridium **sordellii lethal toxin** glucosylation
AUTHOR(S): Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; von Eichel-Streiber, Christoph; Thelestam, Monica; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; et al.
CORPORATE SOURCE: Inst. Pasteur, Unite Toxines Microbiennes, Paris, 75724, Fr.
SOURCE: J. Biol. Chem. (1996), 271(17), 10217-24
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Lethal toxin** (**LT**) from Clostridium **sordellii** is one of the high mol. mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates

Ras, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the **Ras** GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with **LT** prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the **toxin** blocks **Ras** function in vivo. We also demonstrate that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit **Ras** function in vivo.

L5 ANSWER 20 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1996:256001 CAPLUS
DOCUMENT NUMBER: 124:309936
TITLE: Inactivation of **Ras** by Clostridium **sordellii lethal toxin** -catalyzed glucosylation
AUTHOR(S): Just, Ingo; Selzer, Joerg; Hofmann, Fred; Green, Gaynor A.; Aktories, Klaus
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Freiburg, Freiburg, D-79104, Germany
SOURCE: J. Biol. Chem. (1996), 271(17), 10149-53
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **lethal toxin** (**LT**) from Clostridium **sordellii** belongs to the family of large clostridial cytotoxins causing morphol. alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C.. **sordellii LT** exhibits 90% homol. to Clostridium difficile **toxin** B, which has been recently identified as a monoglucosyltransferase (1995). We report here that LT too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low mol. mass GTPases. **LT** selectively modified Rac and **Ras**, whereas the substrate specificity of **toxin** B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both **toxin** B and **LT** share the same acceptor amino acid, threonine 35. Glucosylation of **Ras** by LT results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. **LT** is the first bacterial **toxin** to inactivate **Ras** in intact cells.

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT' ENTERED AT 11:35:20 ON 16 NOV 1999)

Searcher : Shears 308-4994

L6 124 S L5
L7 32 DUP REM L6 (92 DUPLICATES REMOVED)

L7 ANSWER 1 OF 32 TOXLINE
ACCESSION NUMBER: 1999:73084 TOXLINE
DOCUMENT NUMBER: FEDRIP-1999-06409240
TITLE: GTP Binding Proteins in Islet Signal Transduction.
AUTHOR: Kowluru A
CORPORATE SOURCE: Department of Veterans Affairs/Medical Center, Madison, WI
Department of Veterans Affairs/Research and Development (15), 810 Vermont Ave. N.W., Washington, D.C.
CONTRACT NUMBER: VA 00220657
SOURCE: (1999). FEDRIP DATABASE, NATIONAL TECHNICAL INFORMATION SERVICE (NTIS).
FILE SEGMENT: FEDRIP
LANGUAGE: Unavailable
ENTRY MONTH: 199904
AB RPROJ/FEDRIP SIGNAL TRANSDUCTION; PHOSPHOLIPASES; MOLECULAR WEIGHT; G-PROTEINS; EXOCYTOSIS OBJECTIVE: To examine the role of Rho subfamily GTPases in nutrientand calcium-mediated insulin secretion. RESEARCH PLAN AND METHODOLOGY: We utilized Clostridial **toxins** (with known specificities for inhibition of GTPases) to ascertain the contribution of candidate GTPases in physiologic insulin secretion from pancreatic beta cells. FINDINGS: Exposure of normal rat islets or isolated beta (HIT-T15) cells to Clostridium difficile **toxins** A or B catalyzed the glycosylation (and thereby inactivation) of Rac, Cdc42, and Rho endogenous to beta cells; concomitantly, either **toxin** reduced glucose- or potassium-induced insulin secretion from rat islets and HIT cells. Treatment of beta cels with Clostridium **sordellii** **lethal toxin** (**LT**; which modified only **Ras**, Rap and Rac) also reduced glucoseor potassium-induced secretion. However, Clostridial **toxin** C3-exoenzyme (which ADP-ribosylates and inactivates only Rho) was without any effect on either glucoseor potassium-induced insulin secretion. These data suggest that Cdc42, Rac, **Ras** and/or Rap (but not Rho) may be needed for glucoseor potassium-mediated secretion. Effects of these **toxins** appear to be specific on stimulus-secretion coupling, since no difference in metabolic viability (assessed colorimetrically be quantitating the conversion of tetrazolium salt into a formazan in a reduction reaction driven by nutrient metabolism) was demonstrable between control and **toxin** (A or **LT**)-treated beta cells. **Toxin** (A or **LT**)-treatment also did not alter glucoseor potassium-mediated rises in cytosolic free calcium concentrations, suggesting that these GTPases are involved in steps distal to elevation sin cytosolic calcium. Recent findings indicate that the

carboxyl methylation of Cdc42 is stimulated by only glucose, whereas that of Rap and Rac (present study) are regulated by glucose or potassium. SIGNIFICANCE: These provide direct evidence, for the first time, that Rho subfamily of GTPase play key regulatory role(s) in insulin secretion and suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas and Rap and/or Rac may be required for a early steps(s) in 6the stimulus-secretion coupling cascade. These data provide insights into the roles of Rho subfamily GTPases in physiologic insulin secretion which form the basis for our future studies on the metabolic regulation of these proteins in animal models of impaired insulin secretion. Data imply that the CM of gamma subunits in insulin-secreting cells may be facilitated by dissociation of the alpha/beta/gamma trimer into alpha and beta/gamma. Regulation of such a cascade by glucose, an effect dependent on calcium influx and the consequent activation of phospholipases releasing arachidonic acid, implies an important role of the CM of gamma subunits in beta cell function.

L7 ANSWER 2 OF 32 TOXLIT
ACCESSION NUMBER: 1999:68792 TOXLIT
DOCUMENT NUMBER: CA-131-209119P
TITLE: Toxicologically active fragments of **lethal toxin** from Clostridium **sordellii** and their application in immunotoxins.
AUTHOR: Aktories K; Hofmann F
SOURCE: (1999). Ger. Offen. PATENT NO. 19802569 09/09/1999 (Albert-Ludwigs-Universitaet Freiburg). CODEN: GWXXBX.
PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: German
OTHER SOURCE: CA 131:209119
ENTRY MONTH: 199910
AB Fragment 1-546 of C. **sordellii lethal toxin** and an immunotoxin comprising this protein fused to a cell-binding moiety, such as a tumor cell-binding antibody or antibody fragment, are disclosed. The immunotoxin may addnl. contain a translocation signal, e.g., the translocation domain of Pseudomonas exotoxin A or of the Clostridium C2 toxin. The 1-546 fragment of the C. **sordellii lethal toxin** was found to have higher glucosyltransferase activity with **Ras** as substrate than did the wild-type **lethal toxin**.

L7 ANSWER 3 OF 32 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999253957 MEDLINE
DOCUMENT NUMBER: 99253957

TITLE: G-protein-stimulated phospholipase D activity is inhibited by **lethal toxin** from Clostridium **sordellii** in HL-60 cells.
AUTHOR: El Hadj N B; Popoff M R; Marvaud J C; Payrastre B; Boquet P; Geny B
CORPORATE SOURCE: INSERM U332, ICGM, 22 rue Mechain, 75014 Paris, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 14) 274 (20) 14021-31.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199908
ENTRY WEEK: 19990803

AB **Lethal toxin** (**LT**) from Clostridium **sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that **LT** treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with **LT** . A similar dose response to the **toxin** was found when PLD activity was stimulated by phorbol 12-myristate 13-acetate via the protein kinase C pathway. The **toxin** effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota **toxin** from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease observed in phosphatidylinositol 4,5-bisphosphate (PtdIns(4, 5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in **LT**-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. **LT** was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by **LT** and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as **LT toxin** (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, **LT** treatment appeared also to modify cytosol components in relationship with PLD inhibition as a

cytosol prepared from **LT**-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase Calpha was decreased after **LT** treatment. We conclude that in HL-60 cells, **lethal toxin** from C. **sordellii**, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by **LT** in the different modifications observed in HL-60 cells.

L7 ANSWER 4 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1999:307363 SCISEARCH
THE GENUINE ARTICLE: 186VV
TITLE: A novel cytotoxin from Clostridium difficile serogroup F is a functional hybrid between two other large clostridial cytotoxins
AUTHOR: ChavesOlarte E; Low P; Freer E; Norlin T; Weidmann M; vonEichelStreiber C; Thelestam M (Reprint)
CORPORATE SOURCE: KAROLINSKA INST, CTR MICROBIOL & TUMOR BIOL, BOX 280, S-17177 STOCKHOLM, SWEDEN (Reprint); KAROLINSKA INST, CTR MICROBIOL & TUMOR BIOL, S-17177 STOCKHOLM, SWEDEN; UNIV COSTA RICA, UNIDAD MICROSCOPIA ELECT, SAN JOSE, COSTA RICA; KAROLINSKA INST, NOBEL INST NEUROPHYSIOL, DEPT NEUROSCI, S-17177 STOCKHOLM, SWEDEN; UNIV MAINZ, INST MED MIKROBIOL & HYG, VERFUGUNGAGEBAUDE FORSCH & ENTWICKLUNG, D-55101 MAINZ, GERMANY
COUNTRY OF AUTHOR: SWEDEN; COSTA RICA; GERMANY
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (16 APR 1999) Vol. 274, No. 16, pp. 11046-11052.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 21

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The large clostridial cytotoxins (LCTs) constitute a group of high molecular weight clostridial cytotoxins that inactivate cellular small GTP-binding proteins. We demonstrate that a novel LCT (TcdB-1470) from Clostridium difficile strain 1470 is a functional hybrid between ''reference'' TcdB-10463 and Clostridium **sordellii** TcsL-1522. It bound to the same specific receptor

as TcdB-10463 but glucosylated the same GTP-binding proteins as TcsL-1522. Ah three toxins had equal enzymatic potencies but were equally cytotoxic only when micro injected. When applied extracellularly TcdB-1470 and TcdB-10463 were considerably more potent cytotoxins than TcsL-1522. The small GTP-binding protein R-**Ras** was identified as a target for TcdB-1470 and also for Test-1522 but not for TcdB-10463. R-**Ras** is known to control integrin-extracellular matrix interactions from inside the cell. Its glucosylation may be a major determinant for the cell rounding and detachment induced by the two R-**Ras**-attacking toxins. In contrast, fibroblasts treated with TcdB-10463 were arborized and remained attached, with phosphotyrosine containing structures located at the cell-to-cell contacts and beta(3)-integrin remaining at the tips of cellular protrusions. These components were absent from cells treated with the R-**Ras**-inactivating toxins. The novel hybrid toxin will broaden the utility of the LCTs for clarifying the functions of several small GTPases, now including also R-**Ras**.

L7 ANSWER 5 OF 32 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999270964 MEDLINE
DOCUMENT NUMBER: 99270964
TITLE: Effects of cytotoxic necrotizing factor 1 and **lethal toxin** on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers.
AUTHOR: Vouret-Craviari V; Grall D; Flatau G; Pouyssegur J; Boquet P; Van Obberghen-Schilling E
CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, 06108 Nice Cedex 2, France.
SOURCE: INFECTION AND IMMUNITY, (1999 Jun) 67 (6) 3002-8. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199909
ENTRY WEEK: 19990901
AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of **p21** Rho family proteins. In the present study we have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. We report here that Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activates Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induces prominent stress fiber formation without significantly modifying peripheral localization of

VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocks thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (Clostridium botulinum) eliminates actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. **Lethal toxin** (Clostridium **sordellii**), an inhibitor of Rac as well as **Ras** and Rap, potently disrupts the actin microfilament system and monolayer integrity in HUVEC cultures.

L7 ANSWER 6 OF 32 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999383591 MEDLINE
DOCUMENT NUMBER: 99383591
TITLE: Rundown of somatodendritic N-methyl-D-aspartate (NMDA) receptor channels in rat hippocampal neurones: evidence for a role of the small GTPase RhoA.
AUTHOR: Norenberg W; Hofmann F; Illes P; Aktories K; Meyer D K
CORPORATE SOURCE: Department of Pharmacology, Albert-Ludwigs-University, Freiburg, Germany.
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1999 Jul) 127 (5) 1060-3.
Journal code: B00. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY WEEK: 19991202

AB Actin filament (F-actin) depolymerization leads to the use-dependent rundown of N-methyl-D-aspartate (NMDA) receptor activity in rat hippocampal neurones. Depolymerization is promoted by Ca2+ which enters the cells via NMDA receptor channels. The **ras** homologue (Rho) GTPases (RhoA, Rac1 and Cdc42) promote actin polymerization and thus control the actin cytoskeleton. We have investigated, by means of the whole-cell patch clamp technique, whether the actin fibres which interact with NMDA receptors are controlled by Rho GTPases. In the presence of intracellular ATP which attenuates rundown, the C3 toxin from Clostridium (C.) botulinum was used to inactivate RhoA. Indeed, it enhanced the use-dependent rundown of NMDA-evoked inward currents to a level similar to that obtained in the absence of ATP. **Lethal toxin** from Clostridium **sordellii** which inactivates Rac1 and Cdc42 lacked this effect. We suggest that the function of somatodendritic NMDA receptor channels in rat hippocampal neurones can be modulated by RhoA via its action on F-actin.

Searcher : Shears 308-4994

L7 ANSWER 7 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1999:545665 SCISEARCH
THE GENUINE ARTICLE: 214JE
TITLE: The actin-based motility of intracellular Listeria monocytogenes is not controlled by small GTP-binding proteins of the Rho- and **Ras**-subfamilies
AUTHOR: Ebel F (Reprint); Rohde M; vonEichelStreiber C; Wehland J; Chakraborty T
CORPORATE SOURCE: UNIV GIESSEN, INST MED MIKROBIOL, FRANKFURTER STR 107, D-35392 GIESSEN, GERMANY (Reprint); GESELL BIOTECHNOL FORSCH MBH, D-3300 BRAUNSCHWEIG, GERMANY; UNIV MAINZ, INST MED MIKROBIOL & HYG, D-6500 MAINZ, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 JUL 1999) Vol. 176, No. 1, pp. 117-124.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0378-1097.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 24
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this study, we analyzed whether the actin-based motility of intracellular Listeria monocytogenes is controlled by the small GTP-binding proteins of the Rho- and **Ras**-subfamilies. These signalling proteins are key regulatory elements in the control of actin dynamics and their activity is essential for the maintenance of most cellular microfilament structures. We used the Clostridium difficile toxins TcdB-10463 and TcdB-1470 to specifically inactivate these GTP-binding proteins. Treatment of eukaryotic cells with either of these toxins led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the invasion of epithelial cells by L. monocytogenes and had no effect on the actin-based motility of this bacterial parasite. Our data indicate that intracellular Listeria reorganize the actin cytoskeleton in a way that circumvents the control mechanisms mediated by the members of the Rho- and Pas-subfamilies that can be inactivated by the TcdB-10463 and TcdB-1470 toxins. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

L7 ANSWER 8 OF 32 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998344048 MEDLINE
DOCUMENT NUMBER: 98344048
TITLE: A common motif of eukaryotic glycosyltransferases is essential for the enzyme activity of large

clostridial cytotoxins.
AUTHOR: Busch C; Hofmann F; Selzer J; Munro S; Jeckel D; Aktories K
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Hermann-Herder-Str. 5, D-79104 Freiburg, Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jul 31) 273 (31) 19566-72.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199811
ENTRY WEEK: 19981102
AB A fragment of the N-terminal 546 amino acid residues of Clostridium **sordellii lethal toxin** possesses full enzyme activity and glucosylates Rho and **Ras** GTPases in vitro. Here we identified several amino acid residues in C. **sordellii lethal toxin** that are essential for the enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased glucosyltransferase activity by about 5000-fold and completely blocked glucohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of C. **sordellii lethal toxin** was labeled by azido-UDP-glucose after UV irradiation, mutation of the DXD motif prevented radiolabeling. At high concentrations (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced glucosyltransferase activity by about 200-fold and blocked glucohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large number of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.

L7 ANSWER 9 OF 32 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998298120 MEDLINE
DOCUMENT NUMBER: 98298120
TITLE: Functional consequences of monoglucosylation of Ha-**Ras** at effector domain amino acid threonine 35.
AUTHOR: Herrmann C; Ahmadian M R; Hofmann F; Just I
CORPORATE SOURCE: Max-Planck-Institut fur Molekulare Physiologie, Rheinlanddamm 201, D-44139 Dortmund, Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 26) 273

(26) 16134-9.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199810
ENTRY WEEK: 19981002

AB Monoglucosylation of low molecular mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. Ha-**Ras** is monoglucosylated at effector domain amino acid threonine 35 by Clostridium **sordellii lethal toxin**, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the molecular consequences of this modification, effects of glucosylation on each step of the GTPase cycle of **Ras** were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120(GAP) and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated **Ras**. Guanine nucleotide exchange factor (Cdc25)-catalyzed GTP loading was decreased, but not completely inhibited. A dominant-negative property of modified **Ras** to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, **Ras** -effector coupling, was completely blocked. The Kd for the interaction between **Ras**.GTP and the **Ras**-binding domain of Raf was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of **Ras**.GDP for Raf (Kd = 22 &mgr;M) is too low to allow functional interaction, a glucose moiety at threonine 35 of **Ras** seems to block completely the interaction with Raf. The net effect of **lethal toxin**-catalyzed glucosylation of **Ras** is the complete blockade of **Ras** downstream signaling.

L7 ANSWER 10 OF 32 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 1998184846 MEDLINE
DOCUMENT NUMBER: 98184846

TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium **sordellii lethal toxin** and Clostridium difficile toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases.

AUTHOR: Schmidt M; Voss M; Thiel M; Bauer B; Grannass A; Tapp E; Cool R H; de Gunzburg J; von Eichel-Streiber C; Jakobs K H
CORPORATE SOURCE: Institut fur Pharmakologie, Universitatsklinikum Essen, D-45122 Essen, Germany.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 27) 273

(13) 7413-22.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199806
ENTRY WEEK: 19980604

AB Activation of m3 muscarinic acetylcholine receptor (mAChR), stably expressed in human embryonic kidney (HEK)-293 cells, leads to phospholipase D (PLD) stimulation, a process apparently involving Rho GTPases, as shown by studies with Clostridium botulinum C3 exoenzyme and Clostridium difficile toxin B (TcdB). Direct activation of protein kinase C (PKC) by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), also induces PLD stimulation, which is additive to the mAChR action and which is only poorly sensitive to inactivation of Rho proteins by TcdB. To study whether **Ras**-like GTPases are involved in PLD regulation, we studied the effects of the TcdB variant TcdB-1470 and Clostridium **sordellii lethal toxin** (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by mAChR or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concentration-dependent manner, without alteration in immunologically detectable PKC isozyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addition of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addition of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addition of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddition of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L7 ANSWER 11 OF 32 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998147722 MEDLINE
DOCUMENT NUMBER: 98147722
TITLE: Chimeric clostridial cytotoxins: identification of the N-terminal region involved in protein substrate recognition.
AUTHOR: Hofmann F; Busch C; Aktories K
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Germany.
SOURCE: INFECTION AND IMMUNITY, (1998 Mar) 66 (3) 1076-81. Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199805
ENTRY WEEK: 19980502
AB Clostridium **sordellii lethal toxin** is a member of the family of large clostridial cytotoxins that glucosylate small GTPases. In contrast to Clostridium difficile toxins A and B, which exclusively modify Rho subfamily proteins, C. **sordellii lethal toxin** also glucosylates **Ras** subfamily proteins. By deletion analysis and construction of chimeric fusion proteins of C. **sordellii lethal toxin** and C. difficile toxin B, we localized the enzyme activity of the **lethal toxin** to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of C. **sordellii lethal toxin** glucosylated Rho and **Ras** subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of **lethal toxin** for those in the active toxin B fragment (1 to 546) allowed glucosylation of **Ras** subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from **lethal toxin**, and 469 to 546 from toxin B exhibited markedly reduced modification of **Ras** subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of C. **sordellii lethal toxin**

L7 ANSWER 12 OF 32 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1998249799 MEDLINE
DOCUMENT NUMBER: 98249799
TITLE: Rho protein inhibition blocks protein kinase C

translocation and activation.
AUTHOR: Hippenstiel S; Kratz T; Krull M; Seybold J; von Eichel-Streiber C; Suttorp N
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Apr 28) 245 (3) 830-4.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199808
ENTRY WEEK: 19980802
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium **sordellii lethal toxin**-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKC alpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L7 ANSWER 13 OF 32 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1999102800 MEDLINE
DOCUMENT NUMBER: 99102800
TITLE: Inhibition of small G proteins by clostridium **sordellii lethal toxin** activates cdc2 and MAP kinase in Xenopus oocytes.
AUTHOR: Rime H; Talbi N; Popoff M R; Suziedelis K; Jessus C; Ozon R
CORPORATE SOURCE: INRA/ESA-CNRS 7080, Universite Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France.
SOURCE: DEVELOPMENTAL BIOLOGY, (1998 Dec 15) 204 (2) 592-602.
Journal code: E7T. ISSN: 0012-1606.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990401
AB The **lethal toxin** (**LT**) from Clostridium **sordellii** is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. **LT** induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown Xenopus oocytes. **Toxin** B from Clostridium difficile, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras** and/or Rap, negatively regulate cdc2 kinase activation in Xenopus oocyte. In oocyte extracts, **LT** catalyzes the incorporation of [14C]glucose into a group of proteins of 23 kDa and into one protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies, whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of **LT** into oocytes together with UDP-[14C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of **LT**. In vivo time-course analysis reveals that the 27-kDa protein glucosylation is completed within 2 h, well before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of **LT** allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD.

L7 ANSWER 14 OF 32 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 1999168156 MEDLINE
DOCUMENT NUMBER: 99168156
TITLE: **Ras** family proteins: new players involved in the diplotene arrest of Xenopus oocytes.
AUTHOR: Jessus C; Rime H; Ozon R
CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction, Inra/CNRS ESA 7080, Universite Pierre-et-Marie-Curie, Paris, France.
SOURCE: BIOLOGY OF THE CELL, (1998 Nov) 90 (8) 573-83. Ref: 78
Journal code: BOC. ISSN: 0248-4900.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY WEEK: 19990602
AB Oogonia undergo numerous mitotic cell cycles before completing the last DNA replication and entering the meiotic prophase I. After chromosome pairing and chromatid exchanges between paired chromosomes, the oocyte I remains arrested at the diplotene stage of the first meiotic prophase. Oocyte growth then occurs independently of cell division; indeed, during this growth period, oocytes (4n DNA) are prevented from completing the meiotic divisions. How is the prophase arrest regulated? One of the players of the prophase block is the high level of intracellular cAMP, maintained by an active adenylate cyclase. By using **lethal toxin** from Clostridium **sordellii** (**LT**), a glucosyltransferase that glucosylates and inactivates small G proteins of the **Ras** subfamily, we have shown that inhibition of either **Ras** or Rap or both proteins is sufficient to release the prophase block of Xenopus oocytes in a cAMP-dependent manner. The implications of **Ras** family proteins as new players involved in the prophase arrest of Xenopus oocytes will be discussed here.

L7 ANSWER 15 OF 32 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 1999054153 MEDLINE
DOCUMENT NUMBER: 99054153
TITLE: Activation of a Ca2+-dependent K+ current in mouse fibroblasts by lysophosphatidic acid requires a pertussis toxin-sensitive G protein and **Ras**.
AUTHOR: Repp H; Koschinski A; Decker K; Dreyer F
CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Germany.
SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, (1998 Nov) 358 (5) 509-17.
Journal code: NTQ. ISSN: 0028-1298.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990401
AB Lysophosphatidic acid (LPA) is a bioactive lipid that acts through G protein-coupled plasma membrane receptors and mediates a wide range of cellular responses. Here we report that LPA activates a K+ current in NIH3T3 mouse fibroblasts that leads to membrane hyperpolarization. The activation occurs with an EC50 value of 1.7 nM LPA. The K+ current is Ca2+-dependent, voltage-independent, and completely blocked by the K+ channel blockers charybdotoxin, margatoxin, and iberiotoxin with IC50 values of 1.7, 16, and 62 nM,

respectively. The underlying K+ channels possess a single channel conductance of 33 pS in symmetrical K+ solution. Pretreatment of cells with pertussis toxin (PTX), Clostridium **sordellii lethal toxin**, or a farnesyl protein transferase inhibitor reduced the K+ current amplitude in response to LPA to about 25% of the control value. Incubation of cells with the protein tyrosine kinase inhibitor genistein or microinjection of the neutralizing anti-**Ras** monoclonal antibody Y13-259 reduced it by more than 50%. In contrast, the phospholipase C inhibitor U-73122 and the protein kinase A activator 8-bromo-cAMP had no effect. These results indicate that the K+ channel activation by LPA is mediated by a signal transduction pathway involving a PTX-sensitive G protein, a protein tyrosine kinase, and **Ras** . LPA is already known to activate Cl- channels in various cell types, thereby leading to membrane depolarization. In conjunction with our results that demonstrate LPA-induced membrane hyperpolarization by activation of K+ channels, LPA appears to be significantly involved in the regulation of the cellular membrane potential.

L7 ANSWER 16 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1999:475976 SCISEARCH
THE GENUINE ARTICLE: BN14K
TITLE: Activation and inactivation of **Ras**-like GTPases by bacterial cytotoxins
AUTHOR: vonEichelStreiber C (Reprint); Weidmann M; Giry M; Moos M
CORPORATE SOURCE: INST MED MIKROBIOL & HYG, VERFUGUNGSGEBDUDE FORSCH & ENTWICKLUNG, OBERE ZAHLBACHERSTR 63, D-55101 MAINZ, GERMANY (Reprint)
COUNTRY OF AUTHOR: GERMANY
SOURCE: METHODS IN MICROBIOLOGY, (JUN 1998) Vol. 27, pp. 509-525.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL ROAD, LONDON NW1 7DX, ENGLAND.
ISSN: 0580-9517.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 54

L7 ANSWER 17 OF 32 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1998267059 MEDLINE
DOCUMENT NUMBER: 98267059
TITLE: Activation of a Ca2+-dependent K+ current by the oncogenic receptor protein tyrosine kinase v-Fms in mouse fibroblasts.
AUTHOR: Decker K; Koschinski A; Trouliaris S; Tamura T; Dreyer F; Repp H

CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Germany.
SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, (1998 Apr) 357 (4) 378-84.
Journal code: NTQ. ISSN: 0028-1298.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY WEEK: 19981001

AB We investigated the effects of the receptor-coupled protein tyrosine kinase (RTK) v-Fms on the membrane current properties of NIH3T3 mouse fibroblasts. We found that v-Fms, the oncogenic variant of the macrophage colony-stimulating factor receptor c-Fms, activates a K+ current that is absent in control cells. The activation of the K+ current was Ca2+-dependent, voltage-independent, and was completely blocked by the K+ channel blockers charybdotoxin, margatoxin and iberiotoxin with IC50 values of 3 nM, 18 nM and 76 nM, respectively. To identify signalling components that mediate the activation of this K+ current, NIH3T3 cells that express different mutants of the wild-type v-Fms receptor were examined. Mutation of the binding site for the **Ras**-GTPase-activating protein led to a complete abolishment of the K+ current. A reduction of 76% and 63%, respectively, was observed upon mutation of either of the two binding sites for the growth factor receptor binding protein 2. Mutation of the ATP binding lobe, which disrupts the protein tyrosine kinase activity of v-Fms, led to a 55% reduction of the K+ current. Treatment of wild-type v-Fms cells with Clostiridium **sordellii lethal toxin** or a farnesyl protein transferase inhibitor, both known to inhibit the biological function of **Ras**, reduced the K+ current amplitude to 17% and 6% of the control value, respectively. This is the first report showing that an oncogenic RTK can modulate K+ channel activity. Our results indicate that this effect is dependent on the binding of certain **Ras**-regulating proteins to the v-Fms receptor and is not abolished by disruption of its intrinsic protein tyrosine kinase activity. Furthermore, our data suggest that **Ras** plays a key role for K+ channel activation by the oncogenic RTK v-Fms.

L7 ANSWER 18 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:456658 SCISEARCH
THE GENUINE ARTICLE: ZT483
TITLE: Genetic rearrangements in the pathogenicity locus of Clostridium difficile strain 8864 - implications for transcription, expression and enzymatic activity of toxins A and B
AUTHOR: Soehn F; WagenknechtWiesner A; Leukel P; Kohl M;
Searcher : Shears 308-4994

Weidmann M; vonEichelStreiber C (Reprint); Braun V
CORPORATE SOURCE: JOHANNES GUTENBERG UNIV, INST MED MIKROBIOL & HYG, VERFUGUNGSGEBAUDE FORSCH & ENTWICKLUNG, D-55101 MAINZ, GERMANY (Reprint); JOHANNES GUTENBERG UNIV, INST MED MIKROBIOL & HYG, VERFUGUNGSGEBAUDE FORSCH & ENTWICKLUNG, D-55101 MAINZ, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MOLECULAR & GENERAL GENETICS, (MAY 1998) Vol. 258, No. 3, pp. 222-232.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0026-8925.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The pathogenicity locus (PaLoc) of Clostridium difficile isolate 8864 was investigated to locate genetic rearrangements that would explain the exceptional pathogenicity of this particular isolate. Two major changes were defined: an insertion of 1.1 kb between the two genes tcdA and tcdE, coding for the enterotoxin and an accessory protein of unknown function, respectively, and a deletion of 5.9 kb encompassing the 3' ends of tcdA and tcdC. Transcription of the tcdA-E genes is severely affected by both rearrangements, explaining the demonstrated complete lack of TcdA polypeptide. We present a model of coordinate, growth-related transcription of the tcdA-E genes that confirms our previous findings in strain 10463. Recombinant TcdA-8864 had UDP-glucose-glucosyltransferase activity, proving that the N-terminal 698 amino acids of the polypeptide represent the catalytic domain. However, this truncated TcdA molecule lacks a ligand and translocation domain. To assess the catalytic domain of TcdB-8864, the sequence of the 5' end of its gene was determined. TcdB-8864 shows high homology to TcdB-1470 but lower homology to TcdB-10463 within this domain. This fits well with the altered glucosylation specificity of TcdB-8864 (Rac1, Rap2 and Ral). Having defined the variations of transcription, expression and enzymatic activity of toxins A and B, implications for the pathogenic potential of strain 8864 are discussed.

L7 ANSWER 19 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:891246 SCISEARCH
THE GENUINE ARTICLE: 136CD
TITLE: Inhibition of p38 and p42/p44 MAPK by Clostridium **sordellii lethal toxin** in IL-1 stimulated T lymphocytes - A role for a **Ras** subfamily G protein in IL-1 signalling
AUTHOR: Palsson E M (Reprint); Popoff M R; Thelestam M; ONeill L A J

Searcher : Shears 308-4994

CORPORATE SOURCE: TRINITY COLL DUBLIN, DEPT BIOCHEM, DUBLIN, IRELAND; INST PASTEUR, F-75724 PARIS, FRANCE; KAROLINSKA INST, S-17111 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: IRELAND; FRANCE; SWEDEN
SOURCE: EUROPEAN CYTOKINE NETWORK, (SEP 1998) Vol. 9, No. 3, pp. 129-129.
Publisher: JOHN LIBBEY EUROTEXT LTD, 127 AVE DE LA REPUBLIQUE, 92120 MONTROUGE, FRANCE.
ISSN: 1148-5493.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L7 ANSWER 20 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:427521 SCISEARCH
THE GENUINE ARTICLE: ZQ716
TITLE: Signalling through small GTPases
AUTHOR: Mattingly R R (Reprint)
CORPORATE SOURCE: UNIV VIRGINIA, MARKEY CTR CELL SIGNALLING, BOX 577, CHARLOTTESVILLE, VA 22908 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: IN VITRO & MOLECULAR TOXICOLOGY-A JOURNAL OF BASIC AND APPLIED RESEARCH, (SPR 1998) Vol. 11, No. 1, pp. 57-62.
Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON AVENUE, LARCHMONT, NY 10538.
ISSN: 1097-9336.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **Ras** superfamily of small GTPases provides a ubiquitous control mechanism for many cellular processes. Regions outside of the highly-conserved GTPase core domain are variable and show induced conformational changes as the protein cycles between GTP-and GDP-bound states. These sequence variations define several subfamilies of small GTPases that show similar functions. For example, **Ras** proteins control cell growth, Rab proteins direct vesicle fusion, Ran is essential for nuclear protein transport, and Rac/Rho proteins organize the actin cytoskeleton.

Damage to these small GTPases can have catastrophic consequences for the cell and organism. Several Rac/Rho subfamily members are direct targets for clostridial cytotoxins. Further, **Ras** proteins are mutated to a constitutively-active form in approximately 20% of human cancers.

Physiological control of these GTPases switches occurs through exchange factors that catalyze the conversion to the GTP-bound ''on'' state and through GAPs (GTPase-activating proteins) that

accelerate the GTPase activity and the return to the GDP-bound ''off' state. Recent work has identified anew pathway for the activation of **Ras** through phosphorylation of an exchange factor called **Ras**-GRF. Stimulation of receptors coupled to heterotrimeric G-proteins can lead, via a G beta gamma-dependent pathway, to an increase in the specific activity of **Ras** -GRF toward **Ras**.

L7 ANSWER 21 OF 32 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-402313 [37] WPIDS
DOC. NO. CPI: C1997-129748
TITLE: Use of Clostridium **sordellii** **lethal toxin** - for inactivating **Ras** by glucosylation, used for treating conditions such as cancer, particularly pancreatic or colon cancer.
DERWENT CLASS: B04 D16
INVENTOR(S): BOQUET, P; THELESTAM, M; VON EICHELSTREIBER, C; VON EICHEL-STREIBER, C
PATENT ASSIGNEE(S): (BOEF) BOEHRINGER MANNHEIM GMBH; (ASTA) ASTA MEDICA AG
COUNTRY COUNT: 75
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9727871	A1	19970807	(199737)*	EN	45
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN					
AU 9715982	A	19970822	(199801)		
EP 877622	A1	19981118	(199850)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9727871	A1	WO 1997-EP426	19970131
AU 9715982	A	AU 1997-15982	19970131
		WO 1997-EP426	19970131
EP 877622	A1	EP 1997-902278	19970131
		WO 1997-EP426	19970131

FILING DETAILS:

Searcher : Shears 308-4994

PATENT NO	KIND		PATENT NO
AU 9715982	A	Based on	WO 9727871
EP 877622	A1	Based on	WO 9727871

PRIORITY APPLN. INFO: EP 1996-101469 19960202
AN 1997-402313 [37] WPIDS
AB WO 9727871 A UPAB: 19970915
An immunotoxin (A) comprises a first, second and third part, connected by covalent bonds and a pharmaceutically acceptable carrier: (a) the first part includes a target cell specific binding domain, which is able to cause the **LT** (**lethal toxin**) immunotoxin of Clostridium **sordellii** (CS) to bind to the patient's cell; (b) the second part includes a translocation domain of a protein capable of translocating the third part across the cytoplasmic membrane of the cell; and (c) the third part includes a polypeptide with the toxic activity of the catalytic domain of **LT** from CS.

A composition for the treatment of a pathological disorder associated with the activation of **Ras proto**-oncoproteins comprising (A) and a pharmaceutically acceptable carrier is also claimed.

USE - The CS LT can inactivate **Ras** by glucosylation of **Ras** threonine 35. The products can be used for treating cancers, particularly pancrease or colon cancer.
Dwg.0/7

L7 ANSWER 22 OF 32 TOXLIT
ACCESSION NUMBER: 1997:129518 TOXLIT
DOCUMENT NUMBER: CA-127-195467D
TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor.
AUTHOR: Von Eichel-Streiber C; Boquet P; Thelestam M
SOURCE: (1997). PCT Int. Appl. PATENT NO. 9727871 08/07/1997 (Thelestam, Monica).
CODEN: PIXXD2.
PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 127:195467
ENTRY MONTH: 199805
AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras proto**-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium **sordellii toxin LT** under conditions favoring inactivating of **Ras** by glucosylation of **Ras'** threonine 35 in said cell. Said protein preferably is

an immunotoxin which contains as a toxic domain the catalytic domain of **toxin LT**.

L7 ANSWER 23 OF 32 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 97382287 MEDLINE
DOCUMENT NUMBER: 97382287
TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase.
AUTHOR: Fiorentini C; Fabbri A; Flatau G; Donelli G; Matarrese P; Lemichez E; Falzano L; Boquet P
CORPORATE SOURCE: Department of Ultrastructures, Istituto Superiore di Sanit`a, Viale Regina Elena 299, 00161, Rome, Italy.. MD2573@mclink.it
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Aug 1) 272 (31) 19532-7.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199710
ENTRY WEEK: 19971004
AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein **toxin** from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the **toxin** into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent molecular weight of the molecule. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficile **toxin** B, which inactivates Rho but not those of Clostridium **sordellii** **LT toxin**, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-associated phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PIP2) nor the phosphatidylinositol 3,4-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a **toxin** that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L7 ANSWER 24 OF 32 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 1998124099 MEDLINE
DOCUMENT NUMBER: 98124099
TITLE: Evidence for differential roles of the Rho subfamily of GTP-binding proteins in glucose- and calcium-induced insulin secretion from pancreatic beta cells.
AUTHOR: Kowluru A; Li G; Rabaglia M E; Segu V B; Hofmann F; Aktories K; Metz S A
CORPORATE SOURCE: Medical and Research Services, William S. Middleton Memorial VA Medical Center, Madison, WI 53705, USA.. akowluru@facstaff.wisc.edu
CONTRACT NUMBER: DK 37312 (NIDDK)
SOURCE: BIOCHEMICAL PHARMACOLOGY, (1997 Nov 15) 54 (10) 1097-108.
Journal code: 9Z4. ISSN: 0006-2952.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199804
ENTRY WEEK: 19980403
AB We utilized clostridial **toxins** (with known specificities for inhibition of GTPases) to ascertain the contribution of candidate GTPases in physiologic insulin secretion from beta cells. Exposure of normal rat islets or isolated beta (HIT-T15) cells to Clostridium difficile **toxins** A and B catalyzed the glucosylation (and thereby the inactivation) of Rac, Cdc42, and Rho endogenous to beta cells; concomitantly, either **toxin** reduced glucose- or potassium-induced insulin secretion from rat islets and HIT cells. Treatment of beta cells with Clostridium **sordellii lethal toxin** (**LT**; which modified only **Ras**, Rap, and Rac) also reduced glucose- or potassium-induced secretion. However, clostridial **toxin** C3-exoenzyme (which ADP-ribosylates and inactivates only Rho) was without any effect on either glucose- or potassium-induced insulin secretion. These data suggest that Cdc42, Rac, **Ras**, and/or Rap (but not Rho) may be needed for glucose- or potassium-mediated secretion. The effects of these **toxins** appear to be specific on stimulus-secretion coupling, since no difference in metabolic viability (assessed colorimetrically by quantitating the conversion of the tetrazolium salt into a formazan in a reduction reaction driven by nutrient metabolism) was demonstrable between control and **toxin** (A or **LT**)-treated beta cells. **Toxin** (A or **LT**) treatment also did not alter glucose- or potassium-mediated rises in cytosolic free calcium concentrations ([Ca2+]i), suggesting that these GTPases are involved in steps distal to elevations in [Ca2+]i. Recent findings indicate that the carboxyl methylation of Cdc42 is stimulated by only glucose, whereas

that of Rap (Kowluru et al., J Clin Invest 98: 540-555, 1996) and Rac (present study) are regulated by glucose or potassium. Together, these findings provide direct evidence, for the first time, that the Rho subfamily of GTPases plays a key regulatory role(s) in insulin secretion, and they suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas Rap and/or Rac may be required for a later step(s) in the stimulus-secretion coupling cascade (i.e. Ca2+-induced exocytosis of insulin).

L7 ANSWER 25 OF 32 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 96215317 MEDLINE
DOCUMENT NUMBER: 96215317
TITLE: **Ras**, Rap, and Rac small GTP-binding proteins are targets for Clostridium **sordellii lethal toxin** glucosylation.
AUTHOR: Popoff M R; Chaves-Olarte E; Lemichez E; von Eichel-Streiber C; Thelestam M; Chardin P; Cussac D; Antonny B; Chavrier P; Flatau G; Giry M; de Gunzburg J; Boquet P
CORPORATE SOURCE: Institut Pasteur, Unite des Toxines Microbiennes, 75724 Paris, Cedex 15, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 26) 271 (17) 10217-24.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199608

AB **Lethal toxin** (**LT**) from Clostridium **sordellii** is one of the high molecular mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that **LT** is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. **LT** glucosylates **Ras**, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by **LT**. Other related members of the **Ras** GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by **LT**. Incubation of serum-starved Swiss 3T3 cells with **LT** prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the **toxin** blocks **Ras** function in vivo. We also demonstrate that **LT** acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphology. **LT** is thus a powerful tool to inhibit **Ras** function in vivo.

L7 ANSWER 26 OF 32 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 96215306 MEDLINE
DOCUMENT NUMBER: 96215306
TITLE: Inactivation of **Ras** by Clostridium **sordellii lethal toxin** -catalyzed glucosylation.
AUTHOR: Just I; Selzer J; Hofmann F; Green G A; Aktories K
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Universitat Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg, Germany.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 26) 271 (17) 10149-53.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199608
AB The **lethal toxin** (**LT**) from Clostridium **sordellii** belongs to the family of large clostridial cytotoxins causing morphological alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C. **sordellii LT** exhibits 90% homology to Clostridium difficile **toxin** B, which has been recently identified as a monoglucosyltransferase (Just, I., Selzer, J., Wilm, M., von Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375, 500-503). We report here that **LT** too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low molecular mass GTPases. **LT** selectively modifies Rac and **Ras**, whereas the substrate specificity of **toxin** B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both **toxin** B and **LT** share the same acceptor amino acid, threonine 35. Glucosylation of **Ras** by **LT** results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. **LT** is the first bacterial **toxin** to inactivate **Ras** in intact cells.

L7 ANSWER 27 OF 32 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1996:292456 BIOSIS
DOCUMENT NUMBER: PREV199699014812
TITLE: Ca-2+ channel activation by platelet-derived growth factor-induced tyrosine phosphorylation and Ras guanine triphosphate-binding proteins in rat glomerular mesangial cells.
AUTHOR(S): Ma, Heping; Matsunaga, Hiroshi; Li, Bing; Schieffer, Bernhard; Marrero, Mario B.; Ling, Brian N. (1)
CORPORATE SOURCE: (1) Emory Univ. Sch. Med., Renal Div., 1364 Clifton

Road N.E., Atlanta, GA 30322 USA
SOURCE: Journal of Clinical Investigation, (1996) Vol. 97, No. 10, pp. 2332-2341.
ISSN: 0021-9738.
DOCUMENT TYPE: Article
LANGUAGE: English

AB We investigated the signaling pathways mediating 1-pS Ca-2+ channel activation by PDGF in cultured rat mesangial cells. In cell-attached patches, intrapipette PDGF-BB (PDGF B chain homodimer isoform) (50 ng/ml) dramatically stimulates channel activity (P **lt** 0.003, n = 6). Tyrosine kinase inhibition (100 mu-M genistein or 10 mu-M tryphostin 9) abolished PDGF-induced channel activation (P **lt** 0.02, n = 6). In excised patches, the effect of tyrosine kinase inhibition could be reversed by 200 mu-M GTP-gamma-S (P **lt** 0.02, n = 4). In contrast, 200 mu-M GDP-beta-S inhibited PDGF-induced channel activity (P **lt** 0.04, n = 6). Pertussis **toxin** (250 ng/ml) had no effect on PDGF-induced channel activity (P = 0.45, n = 6). When excised patches were exposed to anti-**Ras** antibody (5 mu-g/ ml), PDGF-induced channel activity was abolished (P **lt** 0.002, n = 11). Western immunoblots revealed that PDGF-BB binding stimulates the formation of a membrane-bound complex consisting of growth factor receptor-binding protein 2, son of sevenless, and the PDGF-beta receptor. Complex formation was abolished by genistein. In mesangial cells, the intrinsic tyrosine kinase activity of the PDGF-beta receptor stimulates the formation of a membrane-bound growth factor receptor-binding protein 2/son of sevenless/PDGF-beta receptor complex and activation of the pertussis **toxin**-insensitive GTP-binding protein, **p21-Ras**, which leads to the opening of 1-pS Ca-2+ channels.

L7 ANSWER 28 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 96:754123 SCISEARCH
THE GENUINE ARTICLE: VL996
TITLE: LARGE CLOSTRIDIAL CYTOTOXINS - A FAMILY OF GLYCOSYLTRANSFERASES MODIFYING SMALL GTP-BINDING PROTEINS
AUTHOR: VONEICHELSTREIBER C (Reprint); BOQUET P; SAUERBORN M; THELESTAM M
CORPORATE SOURCE: UNIV MAINZ, INST MED MIKROBIOL & HYG, D-55101 MAINZ, GERMANY (Reprint); FAC MED NICE, INSERM, U452, F-06107 NICE 2, FRANCE; CCLRC DARESBURY LAB, SYNCHROTRON RADIAT DEPT, WARRINGTON WA4 4AD, CHESHIRE, ENGLAND; KAROLINSKA INST, CTR MICROBIOL & TUMORBIOL, S-17177 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: GERMANY; FRANCE; ENGLAND; SWEDEN
SOURCE: TRENDS IN MICROBIOLOGY, (OCT 1996) Vol. 4, No. 10, pp. 375-382.
ISSN: 0966-842X.

DOCUMENT TYPE: Article; Journal
LANGUAGE: ENGLISH
REFERENCE COUNT: 55
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Some Clostridium species produce AB(x)-type protein cytotoxins of high molecular weight. These toxins constitute the group of large clostridial cytotoxins (LCTs), which have homologous protein sequences, exert glycosyltransferase activity and modify GTP-binding proteins of the **Ras**-superfamily. These characteristics render the LCTs valuable tools for developmental and cell biologists.

L7 ANSWER 29 OF 32 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 97127410 MEDLINE
DOCUMENT NUMBER: 97127410
TITLE: Difference in protein substrate specificity between hemorrhagic toxin and **lethal toxin** from Clostridium **sordellii**.
AUTHOR: Genth H; Hofmann F; Selzer J; Rex G; Aktories K; Just I
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Germany.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Dec 13) 229 (2) 370-4.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199703
ENTRY WEEK: 19970304

AB The hemorrhagic toxin (HT) from Clostridium **sordellii** is pharmacologically related to Clostridium difficile toxins A and B and Clostridium **sordellii** **lethal toxin** which have been recently identified as mono-glucosyl-transferases. Here we report that HT, which is coexpressed with **lethal toxin**, is also a glucosyltransferase. Whereas **lethal toxin** glucosylates the Rho subfamily proteins Rac and Cdc42 and the **Ras** subfamily proteins H-**Ras** and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to **lethal toxin**, transferase activity of HT is stimulated by Mn2+. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. **sordellii** HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and **Ras** GTPases.

L7 ANSWER 30 OF 32 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 97011096 MEDLINE

DOCUMENT NUMBER: 97011096
TITLE: The **ras**-related protein Ral is monoglucosylated by Clostridium **sordellii lethal toxin**.
AUTHOR: Hofmann F; Rex G; Aktories K; Just I
CORPORATE SOURCE: Institut fur Pharmakologie and Toxikologie, Albert-Ludwigs-Universitat Freiburg, Germany.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Oct 3) 227 (1) 77-81.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199701
ENTRY WEEK: 19970104
AB Clostridium **sordellii lethal toxin** (**LT**), a cytotoxin which causes preferential destruction of the actin cytoskeleton, has been recently identified as glucosyltransferase to modify the low molecular mass GTPases Rac, **Ras** and Rap. We report here on **LT** produced by C. **sordellii** strain 6018 which glucosylates in addition to Rac, **Ras** and Rap the Ral protein. **LT** from strain VPI9048 however does not glucosylate Ral. Besides recombinant Ral, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equivalent to threonine-35 in H-**Ras** located in the effector region. The Ral-glucosylating **toxin**. is a novel isoform of **Ras**-modifying clostridial cytotoxins.

L7 ANSWER 31 OF 32 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1996:304294 BIOSIS
DOCUMENT NUMBER: PREV199699026650
TITLE: Inactivation of **Ras** by glucosylation catalyzed by Clostridium **sordellii lethal toxin**.
AUTHOR(S): Just, I. (1); Selzer, J. (1); Kern, O. (1); Green, G. A.; Aktories, K. (1)
CORPORATE SOURCE: (1) Inst. Pharmakologie Toxikologie, Univ. Freiburg, D-79104 Freiburg Germany
SOURCE: Naunyn-Schmiedeberg's Archives of Pharmacology, (1996) Vol. 353, No. 4 SUPPL., pp. R19.
Meeting Info.: 37th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Mainz, Germany March 12-14, 1996
ISSN: 0028-1298.
DOCUMENT TYPE: Conference
LANGUAGE: English

L7 ANSWER 32 OF 32 TOXLINE
ACCESSION NUMBER: 1996:71418 TOXLINE
DOCUMENT NUMBER: BIOSIS-96-22459
TITLE: INACTIVATION OF **RAS** BY GLUCOSYLATION CATALYZED BY CLOSTRIDIUM **SORDELLII** **LETHAL TOXIN**.
AUTHOR: JUST I; SELZER J; KERN O; GREEN G A; AKTORIES K
SOURCE: (1996). Vol. 353, No. 4 SUPPL:R19. 37TH SPRING MEETING OF THE GERMAN SOCIETY FOR EXPERIMENTAL AND CLINICAL PHARMACOLOGY AND TOXICOLOGY, MAINZ, GERMANY, MARCH 12-14, 1996. NAUNYN-SCHMIEDEBERG'S ARCHIVES OF PHARMACOLOGY.
CODEN: NSAPCC.
FILE SEGMENT: BIOSIS
LANGUAGE: English
ENTRY MONTH: 199608
AB BIOSIS COPYRIGHT: BIOL ABS. RRM MEETING ABSTRACT
CLOSTRIDIUM-DIFFICILE HUMAN THREONINE

FILE 'USPATFULL' ENTERED AT 11:45:40 ON 16 NOV 1999
L8 2 S L5

L8 ANSWER 1 OF 2 USPATFULL
ACCESSION NUMBER: 1999:24634 USPATFULL
TITLE: Enhancer sequence for modulating expression in epithelial cells
INVENTOR(S): Kufe, Donald, Wellesley, MA, United States
Abe, Miyako, Boston, MA, United States
PATENT ASSIGNEE(S): Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5874415	19990223
APPLICATION INFO.:	US 1995-465981	19950606 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-324465, filed on 17 Oct 1994, now patented, Pat. No. US 5565334 which is a continuation of Ser. No. US 1992-999742, filed on 31 Dec 1992, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Degen, Nancy
LEGAL REPRESENTATIVE: Fish & Richardson P.C.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 863
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated DNA encompassing the DF3 enhancer as well as a sequence

encoding a heterologous polypeptide provides epithelial tissue-selective gene expression of the heterologous polypeptide, useful in methods of therapy.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 514/044.000
INCLS: 514/002.000; 536/024.100; 536/024.500
NCL NCLM: 514/044.000
NCLS: 514/002.000; 536/024.100; 536/024.500

L8 ANSWER 2 OF 2 USPATFULL
ACCESSION NUMBER: 96:94465 USPATFULL
TITLE: Enhancer sequence for modulating expression in epithelial cells
INVENTOR(S): Kufe, Donald, Wellesley, MA, United States
Abe, Miyako, Boston, MA, United States
PATENT ASSIGNEE(S): Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5565334	19961015
APPLICATION INFO.:	US 1994-324465	19941017 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1992-999742, filed on 31 Dec 1992, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Elliott, George C.
LEGAL REPRESENTATIVE: Fish & Richardson P.C.
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 867
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated DNA encompassing the DF3 enhancer as well as a sequence encoding a heterologous polypeptide provides epithelial tissue-selective gene expression of the heterologous polypeptide, useful in methods of therapy.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/069.100
INCLS: 435/240.200; 435/320.100; 536/023.100; 536/023.200; 536/024.100; 536/024.500
NCL NCLM: 435/069.100
NCLS: 435/320.100; 435/371.000; 536/023.100; 536/023.200; 536/024.100; 536/024.500

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT, USPATFULL' ENTERED AT 11:46:17 ON 16 NOV 1999)
Searcher : Shears 308-4994

-Author(s)

L9	20	SEA ABB=ON PLU=ON (EICHEL STREIBER C? OR STREIBER EICHEL C? OR STREIBER C? OR EICHEL C?)/AU
L10	1022	SEA ABB=ON PLU=ON BOQUET P?/AU
L11	728	SEA ABB=ON PLU=ON THELESTAM M?/AU
L12	0	SEA ABB=ON PLU=ON L9 AND L10 AND L11
L13	3	SEA ABB=ON PLU=ON L9 AND (L10 OR L11)
L14	27	SEA ABB=ON PLU=ON L10 AND L11
L15	79	SEA ABB=ON PLU=ON (L9 OR L10 OR L11) AND RAS
L16	93	SEA ABB=ON PLU=ON L13 OR L14 OR L15
L17	24	DUP REM L16 (69 DUPLICATES REMOVED)

L17 ANSWER 1 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1

ACCESSION NUMBER: 1999:351907 CAPLUS

DOCUMENT NUMBER: 131:98722

TITLE: G-protein-stimulated phospholipase D activity is inhibited by lethal toxin from Clostridium sordellii in HL-60 cells

AUTHOR(S): El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud, Jean-Christophe; Payrastre, Bernard; **Boquet, Patrice**; Geny, Blandine

CORPORATE SOURCE: INSERM U332, ICGM, Paris, 75014, Fr.

SOURCE: J. Biol. Chem. (1999), 274(20), 14021-14031
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Lethal toxin (LT) from Clostridium sordellii has been shown in HeLa cells to glucosylate and inactivate **Ras** and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 3-acetate via the protein kinase C pathway. The toxin effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from Clostridium perfringens E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease obsd. in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in Lt-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase

that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as LT toxin (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prepd. from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase C.alpha. was decreased after LT treatment. We conclude that in HL-60 cells, lethal toxin from C. sordellii, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications obsd. in HL-60 cells.

L17 ANSWER 2 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2

ACCESSION NUMBER: 1999:265521 CAPLUS
DOCUMENT NUMBER: 131:40797
TITLE: A novel cytotoxin from Clostridium difficile serogroup F is a functional hybrid between two other large clostridial cytotoxins
AUTHOR(S): Chaves-Olarte, Esteban; Low, Peter; Freer, Enrique; Norlin, Thomas; Weidmann, Manfred; Von Eichel-Streiber, Christoph; **Thelestam, Monica**
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, S-171 77, Swed.
SOURCE: J. Biol. Chem. (1999), 274(16), 11046-11052
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The large clostridial cytotoxins (LCTs) constitute a group of high mol. wt. clostridial cytotoxins that inactivate cellular small GTP-binding proteins. We demonstrate that a novel LCT (TcdB-1470) from Clostridium difficile strain 1470 is a functional hybrid between "ref." TcdB-10463 and Clostridium sordellii TcsL-1522. It bound to the same specific receptor as TcdB-10463 but glucosylated the same GTP-binding proteins as TcsL-1522. All three toxins had equal enzymic potencies but were equally cytotoxic only when

microinjected. When applied extracellularly TcdB-1470 and TcdB-10463 were considerably more potent cytotoxins than TcsL-1522. The small GTP-binding protein R-**Ras** was identified as a target for TcdB-1470 and also for TcsL-1522 but not for TcdB-10463. R-**Ras** is known to control integrin-extracellular matrix interactions from inside the cell. Its glucosylation may be a major determinant for the cell rounding and detachment induced by the two R-**Ras**-attacking toxins. In contrast, fibroblasts treated with TcdB-10463 were arborized and remained attached, with phosphotyrosine contg. structures located at the cell-to-cell contacts and .beta.3-integrin remaining at the tips of cellular protrusions. These components were absent from cells treated with the R-**Ras**-inactivating toxins. The novel hybrid toxin will broaden the utility of the LCTs for clarifying the functions of several small GTPases, now including also R-**Ras**.

L17 ANSWER 3 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
ACCESSION NUMBER: 1999:350413 CAPLUS
DOCUMENT NUMBER: 131:142850
TITLE: Effects of cytotoxic necrotizing factor 1 and lethal toxin on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers
AUTHOR(S): Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; **Boquet, Patrice**; Van Obberghen-Schilling, Ellen
CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, Nice, 06108, Fr.
SOURCE: Infect. Immun. (1999), 67(6), 3002-3008
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study, the authors have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activated Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induced prominent stress fiber formation without modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocked thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme

(Clostridium botulinum) eliminated actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. Lethal toxin (Clostridium sordellii), an inhibitor of Rac as well as **Ras** and Rap, potently disrupted the actin microfilament system and monolayer integrity in HUVEC cultures.

L17 ANSWER 4 OF 24 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:658079 CAPLUS
TITLE: The **Ras** superfamily of small GTP-binding proteins as targets for bacterial toxins
AUTHOR(S): **Boquet, Patrice**
CORPORATE SOURCE: INSERM U452-Faculte de Medecine, Nice, 06107, Fr.
SOURCE: Compr. Sourceb. Bact. Protein Toxins (2nd Ed.) (1999), 27-44. Editor(s): Alouf, Joseph E.; Freer, John H. Academic: London, UK. CODEN: 68GNAV
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Unavailable

L17 ANSWER 5 OF 24 LIFESCI COPYRIGHT 1999 CSA
ACCESSION NUMBER: 1999:21581 LIFESCI
TITLE: UDP-glucose Deficiency Causes Hypersensitivity to the Cytotoxic Effect of Clostridium perfringens Phospholipase C
AUTHOR: Diaz, M.F.; Giron, A.A.; Titball, R.W.; Moos, M.; Guillouard, I.; Cole, S.; Howells, A.M.; **Streiber, C.v.E.**; Florin, I.; **Thelestam, M.**
CORPORATE SOURCE: Microbiology and Tumorbiology Center Karolinska Institutet, S-171 77 Stockholm, Sweden
SOURCE: Journal of Biological Chemistry, (19980918) vol. 273, no. 38, pp. 24433-24438. ISSN: 0021-9258.
DOCUMENT TYPE: Journal
FILE SEGMENT: J
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A Chinese hamster cell line with a mutation in the UDP-glucose pyrophosphorylase (UDPG:PP) gene leading to UDP-glucose deficiency as well as a revertant cell were previously isolated. We now show that the mutant cell is 10 super(5) times more sensitive to the cytotoxic effect of Clostridium perfringens phospholipase C (PLC) than the revertant cell. To clarify whether there is a connection between the UDP-glucose deficiency and the hypersensitivity to C.

perfringens PLC, stable transfectant cells were prepared using a wild type UDPG:PP cDNA. Clones of the mutant transfected with a construct having the insert in the sense orientation had increased their UDP-glucose level, whereas those of the revertant transfected with a UDPG:PP antisense had reduced their level of UDP-glucose compared with control clones transfected with the vector. Exposure of these two types of transfectant clones to C. perfringens PLC demonstrated that a cellular UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of this phospholipase. Further experiments with genetically engineered C. perfringens PLC variants showed that the sphingomyelinase activity and the C-domain are required for its cytotoxic effect in UDP-glucose-deficient cells.

L17 ANSWER 6 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4
ACCESSION NUMBER: 1998:271846 CAPLUS
DOCUMENT NUMBER: 129:52440
TITLE: Rho protein inhibition blocks protein kinase C translocation and activation
AUTHOR(S): Hippenstiel, Stefan; Kratz, Thomas; Krull, Matthias; Seybold, Joachim; **Eichel-Streiber, Christoph V.**; Suttorp, Norbert
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-University, Giessen, D-35392, Germany
SOURCE: Biochem. Biophys. Res. Commun. (1998), 245(3), 830-834
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium sordellii lethal toxin-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as detd. by PKC-activity measurements and Western blots for PKC.alpha.. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated

endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L17 ANSWER 7 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:891246 SCISEARCH
THE GENUINE ARTICLE: 136CD
TITLE: Inhibition of p38 and p42/p44 MAPK by Clostridium sordellii lethal toxin in IL-1 stimulated T lymphocytes - A role for a **Ras** subfamily G protein in IL-1 signalling
AUTHOR: Palsson E M (Reprint); Popoff M R; **Thelestam M**; ONeill L A J
CORPORATE SOURCE: TRINITY COLL DUBLIN, DEPT BIOCHEM, DUBLIN, IRELAND; INST PASTEUR, F-75724 PARIS, FRANCE; KAROLINSKA INST, S-17111 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: IRELAND; FRANCE; SWEDEN
SOURCE: EUROPEAN CYTOKINE NETWORK, (SEP 1998) Vol. 9, No. 3, pp. 129-129.
Publisher: JOHN LIBBEY EUROTEXT LTD, 127 AVE DE LA REPUBLIQUE, 92120 MONTROUGE, FRANCE.
ISSN: 1148-5493.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L17 ANSWER 8 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 5
ACCESSION NUMBER: 1997:533546 CAPLUS
DOCUMENT NUMBER: 127:195467
TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor
INVENTOR(S): Von Eichel-Streiber, Christoph; **Boquet, Patrice**; **Thelestam, Monica**
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany; Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9727871	A1	19970807	WO 1997-EP426	19970131

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR,

KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9715982	A1	19970822	AU 1997-15982	19970131
EP 877622	A1	19981118	EP 1997-902278	19970131

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

PRIORITY APPLN. INFO.:	EP 1996-101469	19960202
	WO 1997-EP426	19970131

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium sordellii toxin LT under conditions favoring inactivating of **Ras** by glucosylation of **Ras'** threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L17 ANSWER 9 OF 24 TOXLIT
ACCESSION NUMBER: 1997:129518 TOXLIT
DOCUMENT NUMBER: CA-127-195467D
TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor.
AUTHOR: Von Eichel-Streiber C; **Boquet P**; **Thelestam M**
SOURCE: (1997). PCT Int. Appl. PATENT NO. 9727871 08/07/1997 (Thelestam, Monica).
CODEN: PIXXD2.
PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF
DOCUMENT TYPE: Patent
FILE SEGMENT: CA
LANGUAGE: English
OTHER SOURCE: CA 127:195467
ENTRY MONTH: 199805

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of Clostridium sordellii toxin LT under conditions favoring inactivating of **Ras** by glucosylation of **Ras'** threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L17 ANSWER 10 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6
ACCESSION NUMBER: 1997:510330 CAPLUS
DOCUMENT NUMBER: 127:172444

TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase

AUTHOR(S): Fiorentini, Carla; Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; **Boquet, Patrice**

CORPORATE SOURCE: Dep. Ultrastructures, Inst. Superiore Sanita, Rome, 00161, Italy

SOURCE: J. Biol. Chem. (1997), 272(31), 19532-19537
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, **Ras**, or Rab6) as demonstrated by a discrete increase in the apparent mol. wt. of the mol. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficult toxin B, which inactivates Rho but not those of Clostridium sordellii LT toxin, which inhibits **Ras** and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-assocd. phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L17 ANSWER 11 OF 24 MEDLINE DUPLICATE 7

ACCESSION NUMBER: 97459997 MEDLINE

DOCUMENT NUMBER: 97459997

TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymatic potencies, cellular substrate specificities, and surface binding to cultured cells.

AUTHOR: Chaves-Olarte E; Weidmann M; **Eichel-Streiber C; Thelestam M**

CORPORATE SOURCE: Microbiology and Tumorbiology Center (MTC), Karolinska institutet, S-171 77 Stockholm, Sweden.

SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1997 Oct 1) 100

(7) 1734-41.
Journal code: HS7. ISSN: 0021-9738.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104

AB Clostridium difficile toxins A and B together are responsible for the symptoms of pseudomembranous colitis. Both toxins intoxicate cultured cells by the same mechanism but they differ in cytotoxic potency, toxin B being generally 1,000 times more potent than toxin A. Don and T84 cells were used to determine differences in the intoxication process exerted by both toxins. Three main differences were identified: (a) the specific binding of radiolabeled toxins to the cell surfaces correlated with the cytotoxic potency, (b) toxin B was found to have a 100-fold higher enzymatic activity than toxin A, and (c) toxin A was found to modify an additional substrate, Rap. The relative contribution of (a) and (b) to the difference in cytotoxic potency was determined by microinjection of the toxins. The differing enzymatic activities turned out to be the main determinant of the difference in cytotoxic potency, whereas the difference in binding contributes to a lesser degree. These findings are discussed in the context of the pathophysiological role of the toxins.

L17 ANSWER 12 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97324529 EMBASE
DOCUMENT NUMBER: 1997324529
TITLE: [The action of active bacterial toxins on p21 Rho G proteins: Its role in cellular regulation].
L'ACTION DES TOXINES BACTERIENNES AGISSANT SUR LA G-PROTEINE P21 RHO: SON ROLE DANS LA REGULATION CELLULAIRE.
AUTHOR: **Boquet P.**; Gauthier M.
CORPORATE SOURCE: P. Boquet, Unite Inserm U452, Faculte de Medecine, Avenue de Valombrose, 06100 Nice Cedex 2, France. u452@unice.fr
SOURCE: Annales de l'Institut Pasteur/Actualites, (1997) 8/2 (173-179).
Refs: 57
ISSN: 0924-4204 CODEN: AIPAEZ
COUNTRY: France
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
048 Gastroenterology
LANGUAGE: French
SUMMARY LANGUAGE: French

L17 ANSWER 13 OF 24 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1997:333400 BIOSIS
DOCUMENT NUMBER: PREV199799632603
TITLE: A mutant cell resistant to Clostridium difficile toxins has a low cytosolic level of UDP-glucose.
AUTHOR(S): Chaves-Olarte, E. (1); Florin, I. (1); **Boquet, P.**; Von Eichel-Streiber, C.; **Thelestam, M.** **(1)**
CORPORATE SOURCE: (1) Microbiol. Tumorbiol. Cent., Karolinska Inst., Box 280, S-171 77 Stockholm Sweden
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1996) Vol. 28, No. 0, pp. 210-211.
Meeting Info.: Seventh European Workshop on Bacterial Protein Toxins Hindsgavl, Denmark July 2-7, 1995
ISSN: 0941-018X.
DOCUMENT TYPE: Book; Conference
LANGUAGE: English

L17 ANSWER 14 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 8
ACCESSION NUMBER: 1996:256012 CAPLUS
DOCUMENT NUMBER: 124:309937
TITLE: **Ras**, Rap, and Rac small GTP-binding proteins are targets for Clostridium sordellii lethal toxin glucosylation
AUTHOR(S): Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; von Eichel-Streiber, Christoph; **Thelestam, Monica**; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; et al.
CORPORATE SOURCE: Inst. Pasteur, Unite Toxines Microbiennes, Paris, 75724, Fr.
SOURCE: J. Biol. Chem. (1996), 271(17), 10217-24
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lethal toxin (LT) from Clostridium sordellii is one of the high mol. mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates **Ras**, Rap, and Rac. In **Ras**, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the **Ras** GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks **Ras** function in vivo. We also demonstrate

that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit **Ras** function in vivo.

L17 ANSWER 15 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 9
ACCESSION NUMBER: 1996:188145 CAPLUS
DOCUMENT NUMBER: 124:223328
TITLE: UDP-glucose deficiency in a mutant cell line protects against glucosyltransferase toxins from Clostridium difficile and Clostridium sordellii
AUTHOR(S): Chaves-Olarte, Esteban; Florin, Inger; **Boquet, Patrice**; Popoff, Michel; von Eichel-Streiber, Christoph; **Thelestam, Monica**
CORPORATE SOURCE: Microbiology & Tumorbiology Center (MTC), Karolinska Inst., Stockholm, S-171 77, Swed.
SOURCE: J. Biol. Chem. (1996), 271(12), 6925-32
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors have previously isolated a fibroblast mutant cell with high resistance to the two Rho-modifying glucosyl-transferase toxins A and B of Clostridium difficile. The authors demonstrate here a low level of UDP-glucose in the mutant, which explains its toxin resistance since: (i) to obtain a detectable toxin B-mediated Rho modification in lysates of mutant cells, addn. of UDP-glucose was required, and it promoted the Rho modification dose-dependently; (ii) high pressure liq. chromatog. anal. of nucleotide exts. of cells indicated that the level of UDP-glucose in the mutant (0.8 nmol/106 cells) was lower than in the wild type (3.7 nmol/106 cells); and (iii) sensitivity to toxin B was restored upon microinjection of UDP-glucose. Using the mutant as indicator cell the authors also found that the related Clostridium sordellii lethal toxin is a glucosyltransferase which requires UDP-glucose as a cofactor. Like toxin B it glucosylated 21-23-kDa proteins in cell lysates, but Rho was not a substrate for lethal toxin.

L17 ANSWER 16 OF 24 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 97055675 MEDLINE
DOCUMENT NUMBER: 97055675
TITLE: Large clostridial cytotoxins--a family of glycosyltransferases modifying small GTP-binding proteins.
AUTHOR: von Eichel-Streiber C; **Boquet P**; Sauerborn M; **Thelestam M**
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie und Hygiene, Johannes Gutenberg-Universitdt Mainz, Germany.. veichel@goofy.zdv.uni.mainz.de
SOURCE: TRENDS IN MICROBIOLOGY, (1996 Oct) 4 (10) 375-82.

Ref: 55
Journal code: B1N. ISSN: 0966-842X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY WEEK: 19970402
AB Some Clostridium species produce ABX-type protein cytotoxins of high molecular weight. These toxins constitute the group of large clostridial cytotoxins (LCTs), which have homologous protein sequences, exert glycosyltransferase activity and modify GTP-binding proteins of the **Ras**-superfamily. These characteristics render the LCTs valuable tools for developmental and cell biologists.

L17 ANSWER 17 OF 24 TOXLINE
ACCESSION NUMBER: 1997:121090 TOXLINE
DOCUMENT NUMBER: BIOSIS-97-22511
TITLE: A MUTANT CELL RESISTANT TO CLOSTRIDIUM DIFFICILE TOXINS HAS A LOW CYTOSOLIC LEVEL OF UDP-GLUCOSE.
AUTHOR: CHAVES-OLARTE E; FLORIN I; **BOQUET P**; VON EICHEL-STREIBER C; **THELESTAM M**
SOURCE: SEVENTH EUROPEAN WORKSHOP ON BACTERIAL PROTEIN TOXINS, HINDSGAVL, DENMARK, JULY 2-7, 1995. ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, (1996). Vol. 28, pp. 210-211.
CODEN: ZBASE2.
FILE SEGMENT: BIOSIS
LANGUAGE: English
ENTRY MONTH: 199709
AB BIOSIS COPYRIGHT: BIOL ABS. RRM BOOK CHAPTER MEETING PAPER CLOSTRIDIUM-DIFFICILE ANIMAL ENZYMOLOGY TOXICOLOGY UDP-GLUCOSE CYTOSOL LOCALIZATION TOXIN A TOXIN B RESISTANCE GLUCOSYL TRANSFERASE RHO SMALL GTPASE ADP-RIBOSYLATION FIBROBLAST CELL BIOLOGY CYTOSOL

L17 ANSWER 18 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 94:203599 SCISEARCH
THE GENUINE ARTICLE: NF017
TITLE: CLOSTRIDIUM-DIFFICILE TOXIN-B ACTS ON THE GTP-BINDING PROTEIN-RHO
AUTHOR: JUST I (Reprint); FRITZ G; AKTORIES K; GIRY M; POPOFF M R; **BOQUET P**; HEGENBARTH S; VONEICHELSTREIBER C
CORPORATE SOURCE: UNIV SAARLAND, INST PHARMACOL & TOXIKOL, D-66421 HOMBURG, GERMANY (Reprint); INST PASTEUR, UNITE TOXINES BACTERIENNES, F-75724 PARIS 15, FRANCE; UNIV

MAINZ, INST MED MICROBIOL, D-55101 MAINZ, GERMANY
COUNTRY OF AUTHOR: GERMANY; FRANCE
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (08 APR 1994) Vol. 269, No. 14, pp. 10706-10712.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Clostridium difficile toxin B exhibits cytotoxic activity that is characterized by the disruption of the microfilamental cytoskeleton. Here we studied whether the GTP-binding Rho protein, which reportedly participates in the regulation of the actin cytoskeleton, is involved in the toxin action. Toxin B treatment of Chinese hamster ovary cells reveals a time- and concentration-dependent decrease in the ADP-ribosylation of Rho by Clostridium botulinum C3 exoenzyme in the cell lysate. Disruption of the microfilament system induced by C. botulinum C2 toxin or cytochalasin D does not cause impaired ADP-ribosylation of Rho. Toxin B exhibits its effects on Rho not only in intact cells but also when added to cell lysates. Besides endogenous Rho, RhoA-glutathione S-transferase (Rho-GST) fusion protein added to cell lysate showed decreased ADP-ribosylation after toxin B treatment. Immunoblot analysis reveals identical amounts of Rho-GST and no change in molecular mass after toxin B treatment compared with controls. ADP-ribosylation of Rho-GST purified from toxin B-treated cell lysate is inhibited, indicating a modification of Rho itself. Finally, transfection of rhoA DNA under the control of a strong promoter into cells protects them from the activity of toxin B. Altogether, the data indicate that C. difficile toxin B acts directly or indirectly on Rho proteins to inhibit ADP-ribosylation and suggest that the cytotoxic effect of toxin B involves Rho.

L17 ANSWER 19 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 94:253931 SCISEARCH
THE GENUINE ARTICLE: NJ034
TITLE: CYTOTOXIC NECROTIZING FACTOR TYPE-2 PRODUCED BY VIRULENT ESCHERICHIA-COLI MODIFIES THE SMALL GTP-BINDING PROTEINS-RHO INVOLVED IN ASSEMBLY OF ACTIN STRESS FIBERS
AUTHOR: OSWALD E; SUGAI M; LABIGNE A; WU H C; FIORENTINI C; **BOQUET P**; OBRIEN A D (Reprint)
CORPORATE SOURCE: UNIFORMED SERV UNIV HLTH SCI, DEPT MICROBIOL & IMMUNOL, BETHESDA, MD, 20814 (Reprint); UNIFORMED SERV UNIV HLTH SCI, DEPT MICROBIOL & IMMUNOL, BETHESDA, MD, 20814; HIROSHIMA UNIV, SCH DENT, DEPT MICROBIOL, HIROSHIMA 734, JAPAN; INST PASTEUR, INSERM, U193, UNITE ENTEROBACTERIES, F-75015 PARIS,

other cells rapidly underwent temporary morphol. alterations that were in certain respects similar to those seen after microinjection of cloned gene **ras** proteins. When injected into Xenopus oocytes, C3 induced migration of germinal vesicles and potentiated the cholera toxin-sensitive augmentation of germinal vesicle breakdown by progesterone, also as caused by **ras** proteins. Nevertheless, p21.bot was immunol. distinct from p21ras.

L17 ANSWER 24 OF 24 LIFESCI COPYRIGHT 1999 CSA
ACCESSION NUMBER: 86:71890 LIFESCI
TITLE: Structure/function relationships of tetanus toxin. A comparison with the diphtheria toxin molecule. BACTERIAL PROTEIN TOXINS.
AUTHOR: Roa, M.; Kagan, B.L.; **Boquet, P.**; Falmagne, P. [editor]; Alouf, J.E. [editor]; Fehrenbach, F.J. [editor]; Jeljaszewicz, J. [editor]; **Thelestam, M.** [editor]
CORPORATE SOURCE: Unite Antigenes Bact. (UA CNRS 040557), Inst. Pasteur, 75724 Paris Cedex 15, France
SOURCE: ZENTRALBL. BAKTERIOL. MIKROBIOL. HYG., (1986) pp. 27-32.
Meeting Info.: 2. European Workshop on Bacterial Protein Toxins. Wepion (Belgium). 30 Jun-4 Jul 1985.
ISBN: 3-437-11083-7.
DOCUMENT TYPE: Book
TREATMENT CODE: Conference
FILE SEGMENT: J; X
LANGUAGE: English

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT, USPATFULL' ENTERED AT 11:46:17 ON 16 NOV 1999)
L18 400 S VON EICHEL ?/AU
L19 109 S L18 AND (RAS OR L10 OR L11)
L20 76 S L19 NOT L16
L21 18 DUP REM L20 (58 DUPLICATES REMOVED)

L21 ANSWER 1 OF 18 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:658085 CAPLUS
TITLE: Clostridial toxins acting on the cytoskeleton
AUTHOR(S): **Thelestam, Monica**; Chaves-Olarte, Esteban; Moos, Michael; **Von Eichel-Streiber, Christoph**
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, S-17177, Swed.
SOURCE: Compr. Sourceb. Bact. Protein Toxins (2nd Ed.) (1999), 147-173. Editor(s): Alouf, Joseph E.; Freer, John H. Academic: London, UK.
CODEN: 68GNAV
Searcher : Shears 308-4994

DOCUMENT TYPE: Conference
LANGUAGE: English
AB Unavailable

L21 ANSWER 2 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
ACCESSION NUMBER: 1999:486236 CAPLUS
DOCUMENT NUMBER: 131:211505
TITLE: The actin-based motility of intracellular Listeria monocytogenes is not controlled by small GTP-binding proteins of the rho- and **Ras**-subfamilies
AUTHOR(S): Ebel, Frank; Rohde, Manfred; **Von Eichel-Streiber, Christoph**; Wehland, Jurgen; Chakraborty, Trinad
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, 35392, Germany
SOURCE: FEMS Microbiol. Lett. (1999), 176(1), 117-124
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, the authors analyzed whether the actin-based motility of intracellular Listeria monocytogenes is controlled by the small GTP-binding proteins of the Rho- and **Ras**-subfamilies. These signalling proteins are key regulatory elements in the control of actin dynamics and their activity is essential for the maintenance of most cellular microfilament structures. The authors used the Clostridium difficile toxins TcdB-10463 and TcdB-1470 to specifically inactivate these GTP-binding proteins. Treatment of eukaryotic cells with either of these toxins led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the invasion of epithelial cells by L. monocytogenes and had no effect on the actin-based motility of this bacterial parasite. The data indicate that intracellular Listeria reorganize the actin cytoskeleton in a way that circumvents the control mechanisms mediated by the members of the Rho- and **Ras**-subfamilies that can be inactivated by the TcdB-10463 and TcdB-1470 toxins.

L21 ANSWER 3 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
ACCESSION NUMBER: 1998:638922 CAPLUS
DOCUMENT NUMBER: 130:21586
TITLE: UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of Clostridium perfringens phospholipase C
AUTHOR(S): Flores-Diaz, Marietta; Alape-Giron, Alberto; Titball, Richard W.; Moos, Michael; Guillouard, Isabelle; Cole, Stewart; Howells, Angela M.; **Von Eichel-Streiber, Christoph**; Florin,

Inger; **Thelestam, Monica**
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, S-171 77, Swed.
SOURCE: J. Biol. Chem. (1998), 273(38), 24433-24438
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A Chinese hamster cell line with a mutation in the UDP-glucose pyrophosphorylase (UDPG:PP) gene leading to UDP-glucose deficiency as well as a revertant cell were previously isolated. The authors now show that the mutant cell is 105 times more sensitive to the cytotoxic effect of Clostridium perfringens phospholipase C (PLC) than the revertant cell. To clarify whether there is a connection between the UDP-glucose deficiency and the hypersensitivity to C. perfringens PLC, stable transfectant cells were prepd. using a wild type UDPG:PP cDNA. Clones of the mutant transfected with a construct having the insert in the sense orientation had increased their UDP-glucose level, whereas those of the revertant transfected with a UDPG:PP antisense had reduced their level of UDP-glucose compared with control clones transfected with the vector. Exposure of these two types of transfectant clones to C. perfringens PLC demonstrated that a cellular UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of this phospholipase. Further expts. with genetically engineered C. perfringens PLC variants showed that the sphingomyelinase activity and the C-domain are required for its cytotoxic effect in UDP-glucose-deficient cells.

L21 ANSWER 4 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:223590 CAPLUS
DOCUMENT NUMBER: 128:318211
TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium sordellii lethal toxin and Clostridium difficile toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases
AUTHOR(S): Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; De Gunzburg, Jean; **Von Eichel-Streiber, Christoph**; Jakobs, Karl H.
CORPORATE SOURCE: Universitatsklinikum Essen, Institut fur Pharmakologie, Essen, D-45122, Germany
SOURCE: J. Biol. Chem. (1998), 273(13), 7413-7422
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
Searcher : Shears 308-4994

AB To study whether **Ras**-like GTPases are involved in phospholipase D (PLD) regulation, we studied the effects of the Clostridium difficile toxin B (TcdB) variant TcdB-1470 and Clostridium sordellii lethal toxin (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by m3 muscarinic acetylcholine receptor (mAChR) or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time-and concn.-dependent manner, without alteration in immunol. detectable protein kinase C (PKC) isoenzyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addn. of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addn. of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addn. of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TscL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddn. of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L21 ANSWER 5 OF 18 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 1998249799 MEDLINE
DOCUMENT NUMBER: 98249799
TITLE: Rho protein inhibition blocks protein kinase C translocation and activation.
AUTHOR: Hippenstiel S; Kratz T; Krull M; Seybold J; **von Eichel-Streiber C**; Suttorp N
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Apr 28) 245 (3) 830-4.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
Searcher : Shears 308-4994

LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199808
ENTRY WEEK: 19980802
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium sordellii lethal toxin-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKC alpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L21 ANSWER 6 OF 18 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:6411 CAPLUS
DOCUMENT NUMBER: 130:221101
TITLE: Activation and inactivation of **ras** -like GTPases by bacterial cytotoxins
AUTHOR(S): **Von Eichel-Streiber, Christoph**; Weidmann, Manfred; Giry, Murielle; Moos, Michael
CORPORATE SOURCE: Verfugungsgebaude fur Forschung und Entwicklung, Institut fur Medizinische Mikrobiologie und Hygiene, Mainz, D-55101, Germany
SOURCE: Methods Microbiol. (1998), 27(Bacterial Pathogenesis), 509-525
CODEN: MMICEU; ISSN: 0580-9517
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 54 refs. Topics include: modulation of the eukaryotic skeleton by bacterial products; cytoskeletal targets of bacterial toxins; the switch characteristic of GTPases; Rho-modulating bacterial toxins; **Ras**-modulating bacterial factors; use of LCTs as tools in cell biol. (c) 1998 Academic Press.

L21 ANSWER 7 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 5
ACCESSION NUMBER: 1998:459143 BIOSIS

DOCUMENT NUMBER: PREV199800459143
TITLE: A UDP-glucose deficient cell mutant as model to study the molecular mechanism of cytotoxicity induced by C. perfringens phospholipase C in ischemic tissue.
AUTHOR(S): **Thelestam, M.** (1); Flores Dfaz, M. (1); Alape Giron, A. (1); Titball, R.; Pollesello, P.; Persson, B.; Moos, M.; Chaves Olarte, E. (1); Lofrumento, D.; Cortes Bratii, X. (1); Bergman, T.; **Von Eichel-Streiber, C.**; Florin, I. (1)
CORPORATE SOURCE: (1) Microbiol. and Tumorbiol. Cent., Karolinska Inst., S-171 77 Stockholm Sweden
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1998) Vol. 29, pp. 184-191.
Meeting Info.: Eighth European Workshop on Bacterial Protein Toxins Staffelstein, Kloster Banz, Germany June 29-July 4, 1997
ISSN: 0941-018X.
DOCUMENT TYPE: Conference
LANGUAGE: English

L21 ANSWER 8 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6
ACCESSION NUMBER: 1998:397225 CAPLUS
DOCUMENT NUMBER: 129:134470
TITLE: Small GTP-binding proteins of the Rho- and **Ras**-subfamilies are not involved in the actin rearrangements induced by attaching and effacing Escherichia coli
AUTHOR(S): Ebel, Frank; **von Eichel-Streiber, Christoph**; Rohde, Manfred; Chakraborty, Trinad
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, D-35392, Germany
SOURCE: FEMS Microbiol. Lett. (1998), 163(2), 107-112
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Attaching and effacing Escherichia coli (AEEC) are extracellular pathogens that induce the formation of actin-rich structures at their sites of attachment to eukaryotic host cells. We analyzed whether small GTP-binding proteins of the Rho- and **Ras**-subfamilies, which control the cellular actin system, are essential for these bacterial-induced microfilament reorganizations. For this purpose we specifically inactivated them using the Clostridium difficile toxins TcdB-10463 and TcdB-1470. Such treatment led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the bacterial-induced actin rearrangements. Our data therefore indicate that the microfilament reorganizations induced by

AEEC are independent of those small GTP-binding proteins that under normal conditions control the dynamics and maintenance of the actin cytoskeleton.

L21 ANSWER 9 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 7
ACCESSION NUMBER: 1998:459113 BIOSIS
DOCUMENT NUMBER: PREV199800459113
TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymatic potencies, cellular substrate specificities and surface binding to cultured cells.
AUTHOR(S): Chaves-Olarte, E. (1); Weidmann, M.; **Von Eichel-Streiber, C.; Thelestam, M. (1)**
CORPORATE SOURCE: (1) Microbiol. and Tumorbiol. Cent., Karolinska Inst., Stockholm Sweden
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1998) Vol. 29, pp. 74-75.
Meeting Info.: Eighth European Workshop on Bacterial Protein Toxins Staffelstein, Kloster Banz, Germany June 29-July 4, 1997
ISSN: 0941-018X.
DOCUMENT TYPE: Conference
LANGUAGE: English

L21 ANSWER 10 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:293569 BIOSIS
DOCUMENT NUMBER: PREV199800293569
TITLE: Role of Ral GTPases in protein kinase C-induced phospholipase D stimulation.
AUTHOR(S): Voss, M. (1); Bauer, B.; Cool, R. H.; **Von Eichel-Streiber, C.**; Jakobs, K. H. (1); Schmidt, M. (1)
CORPORATE SOURCE: (1) Inst. Pharmakol., Univ. GH Essen, D-45122 Essen Germany
SOURCE: Naunyn-Schmiedeberg's Archives of Pharmacology, (1998) Vol. 357, No. 4 SUPPL, pp. R56.
Meeting Info.: 39th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Mainz, Germany March 17-19, 1998 German Society for Experimental and Clinical Pharmacology and Toxicology
. ISSN: 0028-1298.
DOCUMENT TYPE: Conference
LANGUAGE: English

L21 ANSWER 11 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 8
ACCESSION NUMBER: 1997:637149 CAPLUS
DOCUMENT NUMBER: 127:329879
TITLE: Cellular UDP-glucose deficiency caused by a single point mutation in the UDP-glucose

pyrophosphorylase gene

AUTHOR(S): Flores-Diaz, Marietta; Alape-Giron, Alberto; Persson, Bengt; Pollesello, Piero; Moos, Michael; **von Eichel-Streiber, Christoph**; **Thelestam, Monica**; Florin, Inger

CORPORATE SOURCE: Microbiology and Tumorbiology Center and Dep. of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, S-171 77, Swed.

SOURCE: J. Biol. Chem. (1997), 272(38), 23784-23791
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors previously isolated a mutant cell that is the only mammalian cell reported to have a persistently low level of UDP-glucose. In this work the authors obtained a spontaneous revertant whose UDP-glucose level lies between those found in the wild type and the mutant cell. The activity of UDP-glucose pyrophosphorylase (UDPG:PP), the enzyme that catalyzes the formation of UDP-glucose, was in the mutant 4% and in the revertant 56% of the activity found in the wild type cell. Sequence anal. of UDPG: PP cDNAs from the mutant cell showed one missense mutation, which changes amino acid residues 115 from glycine to aspartic acid. The substituted glycine is located within the largest stretch of strictly conserved residues among eukaryotic UDPG:PPs. The anal. of the cDNAs from the revertant cell indicated the presence of an equimolar mixt. of the wild type and the mutated mRNAs, suggesting that the mutation has reverted in only one of the alleles. In summary, the authors demonstrate that the G115D substitution in the Chinese hamster UDPG:PP dramatically impairs its enzymic activity, thereby causing cellular UDP-glucose deficiency.

L21 ANSWER 12 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 9

ACCESSION NUMBER: 1997:653189 CAPLUS

DOCUMENT NUMBER: 127:274019

TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymic potencies, cellular substrate specificities, and surface binding to cultured cells

AUTHOR(S): Chaves-Olarte, Esteban; Weidmann, Manfred; **Von Eichel-Streiber, Christoph**; **Thelestam, Monica**

CORPORATE SOURCE: Microbiology and Tumorbiology Center (MTC), Karolinska institutet, Stockholm, S-171 77, Swed.

SOURCE: J. Clin. Invest. (1997), 100(7), 1734-1741
CODEN: JCINAO; ISSN: 0021-9738

PUBLISHER: Rockefeller University Press

DOCUMENT TYPE: Journal
LANGUAGE: English
AB C. difficile toxins A and B together are responsible for the symptoms of pseudomembranous colitis. Both toxins intoxicate cultured cells by the same mechanism but they differ in cytotoxic potency, toxin B being generally 1000 times more potent than toxin A. Don and T84 cells were used to det. differences in the intoxication process exerted by both toxins. Three main differences were identified: (1) the specific binding of radiolabeled toxins to the cell surfaces correlated with the cytotoxic potency, (2) toxin B was found to have a 100-fold higher enzymic activity than toxin A, and (3) toxin A was found to modify an addnl. substrate, Rap. The relative contribution of (1) and (2) to the difference in cytotoxic potency was detd. by microinjection of the toxins. The differing enzymic activities turned out to be the main determinant of the difference in cytotoxic potency whereas the difference in binding contributes to a lesser degree. These findings are discussed in the context of the pathophysiol. role of the toxins.

L21 ANSWER 13 OF 18 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:419486 CAPLUS
DOCUMENT NUMBER: 129:187684
TITLE: A UDP-glucose deficient cell mutant as model to study the molecular mechanism of cytotoxicity induced by C. perfringens phospholipase C in ischemic tissue
AUTHOR(S): **Thelestam, M.**; Flores Dfaz, M.; Alape Giron, A.; Titball, R.; Pollesello, P.; Persson, B.; Moos, M.; Chaves Olarte, E.; Lofrumento, D.; Cortes Bratii, X.; Bergman, T.; **Von Eichel-Streiber, C.**; Florin, I.
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, S-171 77, Swed.
SOURCE: Zentralbl. Bakteriol., Suppl. (1997), 29(Bacterial Protein Toxins), 184-191
CODEN: ZBASE2; ISSN: 0941-018X
PUBLISHER: Gustav Fischer Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A Chinese hamster mutant cell line has a UDPG deficiency conferring resistance to glucosyltransferase toxins. The deficiency is caused by a point mutation in the UDPG:pyrophosphorylase (UDPG:PP) gene, as shown by sequence anal. and confirmed by transfection expts. The mutant cell overproduces calreticulin (CRT) and four glucose-regulated stress proteins (GRPs) and is hypersensitive to C. perfringens phospholipase C (PLC). Wild type cells subjected to glucose starvation exhibit a UDPG deficiency, up-regulate CRT and GRPs and are also hypersensitive to PLC. Transfection of the mutant cell with bovine UDPG:PP cDNA demonstrated a linkage between the

UDPG deficiency and the hypersensitivity to PLC. The mutant cell will be a useful model to clarify the mol. mechanism of the cytotoxicity induced by PLC as well as the role of UDPG for mol. signaling in cells under glucose starvation.

L21 ANSWER 14 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 10
ACCESSION NUMBER: 1997:420507 CAPLUS
DOCUMENT NUMBER: 127:77178
TITLE: Delineation of the catalytic domain of Clostridium difficile toxin B-10463 to an enzymically active N-terminal 467 amino acid fragment
AUTHOR(S): Wagenknecht-Wiesner, Alice; Weidmann, Manfred; Braun, Veit; Leukel, Petra; Moos, Michael; **von Eichel-Streiber, Christoph**
CORPORATE SOURCE: Verfuegungsgebaeude fuer Forschung und Entwicklung, Institut fuer medizinische Mikrobiologie und Hygiene, Johannes Gutenberg-Universitaet, Obere Zahlbacherstr. 63, Mainz, 55101, Germany
SOURCE: FEMS Microbiol. Lett. (1997), 152(1), 109-116
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To directly approach the postulated toxic domain of Clostridium difficile's TcdB-10463, eight subclones of different size and locations in the N-terminal third of the toxin were generated. Expression of these toxin fragments was checked in Western blots and the enzymic activity of the expressed proteins was analyzed by glucosylating **Ras** related small GTP-binding proteins. Two polypeptides of 875 aa (TcdBc1-3) and 557 aa (TcdBc1-H) glucosylated their targets Rho, Rac and Cdc42 with the same activity and specificity as the holotoxin. In comparison 516 aa (TcdBc1-N) and 467 aa (TcdBc1-A) protein fragments exhibited highly reduced activity, while Tcdc1 and TcdB2-3 (aa 1-243 and 244-890, resp.) were enzymically inactive. Our results indicate that all structures involved in the catalysis are located at several different sites within the 557 aa fully active fragment. The shortest enzymically still active protein covers aa 1-467 and obviously fulfills all minimal requirements for glucosylation. The data support the postulated three domain model of 'large clostridial cytotoxins'.

L21 ANSWER 15 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 11
ACCESSION NUMBER: 1995:628937 CAPLUS
DOCUMENT NUMBER: 123:27527
TITLE: The enterotoxin from Clostridium difficile (ToxA) monoglucosylates the Rho proteins
AUTHOR(S): Just, Ingo; Wilm, Matthias; Selzer, Joerg; Rex,

Gundula; **von Eichel-Streiber, Christoph** ; Mann, Matthias; Aktories, Klaus
CORPORATE SOURCE: Institut Pharmakologie, Toxikologie, Universitat Saarlandes, Hamburg/Saar, D-66421, Germany
SOURCE: J. Biol. Chem. (1995), 270(23), 13932-6
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Here we report on the identification of the enterotoxin from Clostridium difficile (ToxA)-induced modification of Rho proteins. From several hexoses tested UDP-glucose selectively served as cosubstrate for ToxA-catalyzed modification. The acceptor amino acid of glucosylation was identified from a Lys-C-generated peptide by tandem mass spectrometry as Thr-37. mutation of Thr-37 to Ala completely abolished glucosylation. The members of the Rho family (RhoA, Rac1, and Cdc42Hs) were substrates for ToxA, whereas H-**Ras**, Rab5, and Arf1 were not glucosylated. ToxA-catalyzed glucosylation of lysates from ToxA-pretreated rat basophilic leukemia (RBL) cells resulted in a decreased incorporation of [14C]glucose, indicating previous glucosylation in the intact cell. Glucosylation of the Rho subtype proteins appears to be the mol. mechanism by which C. difficile ToxA mediates its cytotoxic effects on cells.

L21 ANSWER 16 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 12
ACCESSION NUMBER: 1995:832244 CAPLUS
DOCUMENT NUMBER: 123:224757
TITLE: Transient expression of RhoA, -B, and -C GTPases in HeLa cells potentiates resistance to Clostridium difficile toxins A and B but not to Clostridium sordellii lethal toxin
AUTHOR(S): Giry, Murielle; Popoff, Michel R.; **von Eichel-Streiber, Christoph; Boquet, Patrice**
CORPORATE SOURCE: Unite des Toxines Microbiennes, Institut Pasteur, Paris, 75724, Fr.
SOURCE: Infect. Immun. (1995), 63(10), 4063-71
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The bacterial pathogen Clostridium difficile synthesizes two high-mol.-wt. toxins (A and B), which exhibit toxic effects in vivo and in vitro. Here, the authors present evidence that the major intracellular targets of these two toxins are the Rho GTPases. Overexpression of RhoA, RhoB, or RhoC GTPases in transfected HeLa cells conferred an increased resistance to toxins A and B, indicating that these toxins cause their cytopathic effects primarily by affecting Rho proteins. In addn., toxin A and B treatment appeared to result in modification of Rho, since Rho

isolated from toxin-treated cells had a decreased ability to be ADP-ribosylated by Clostridium botulinum C3 exoenzyme. In contrast, the lethal toxin (LT) of Clostridium sordellii, although structurally and immunol. related to C. difficile toxin B, appeared to induce cytopathic effects independently of the Rho GPTases. Overexpression of RhoA in transfected HeLa cells did not protect them from the effect of LT, and Rho isolated from lysates of LT-treated cells was not resistant to modification by C3. Immunofluorescence studies showed that LT treatment caused a cytopathic effect that was very different from those described for C. difficile toxins A and B, resulting in an increase in cortical F-actin, which a concomitant decrease in the no. of stress fibers, and in the formation of numerous microvilli contg. the actin-bundling protein fimbrin/plastin.

L21 ANSWER 17 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 13
ACCESSION NUMBER: 1996:44030 BIOSIS
DOCUMENT NUMBER: PREV199698616165
TITLE: Effects of Clostridium difficile toxins A and B and the lethal toxin of Clostridium sordellii on the Rho GTPases.
AUTHOR(S): **Boquet, P. (1)**; Popoff, M. R. (1); **Von Eichel-Streiber, C.**; Giry, M. (1)
CORPORATE SOURCE: (1) Unite Toxines Microbiennes, Inst. Pasteur, 28 rue du Docteur Roux, 75724 Paris, Cedex 15 France
SOURCE: Microbial Ecology in Health and Disease, (1995) Vol. 8, No. 4, pp. 191.
Meeting Info.: Workshop on Recent Advances in Clostridium difficile and its Toxins Tours, France May 4, 1995
ISSN: 0891-060X.
DOCUMENT TYPE: Conference
LANGUAGE: English

L21 ANSWER 18 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 14
ACCESSION NUMBER: 1994:291810 CAPLUS
DOCUMENT NUMBER: 120:291810
TITLE: Clostridium difficile toxin B acts on the GTP-binding protein Rho
AUTHOR(S): Just, Ingo; Fritz, Gerhard; Aktories, Klaus; Giry, Murielle; Popoff, Michel R.; **Boquet, Patrice**; Hegenbarth, Silke; **von Eichel-Streiber, Christoph**
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Saarlandes, Homburg-Saar, D-66421, Germany
SOURCE: J. Biol. Chem. (1994), 269(14), 10706-12
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

Searcher : Shears 308-4994

AB C. difficile toxin B exhibits cytotoxic activity that is characterized by the disruption of the microfilamental cytoskeleton. Here the authors studied whether the GTP-binding Rho protein, which reportedly participates in the regulation of the actin cytoskeleton, is involved in the toxin action. Toxin B treatment of Chinese hamster ovary cells reveals a time- and concn.-dependent decrease in the ADP-ribosylation of Rho by Clostridium botulinum C3 exoenzyme in the cell lyzate. Disruption of the microfilament system induced by C. botulinum C2 toxin or cytochalasin D does not cause impaired ADP-ribosylation of Rho. Toxin B exhibits its effects on Rho not only in intact cells but also when added to cell lyzates. Besides endogenous Rho, RhoA-glutathione S-transferase (Rho-GST) fusion protein added to cell lysate showed decreased ADP-ribosylation after toxin B treatment. Immunoblot anal. reveals identical amts. of Rho-GST and no change in mol. mass after toxin B treatment compared with controls. ADP-ribosylation of Rho-GST purified from toxin B-treated cell lyzate is inhibited, indicating a modification of Rho itself. Finally, transfection of rhoA DNA under the control of a strong promoter into cells protects them from the activity of toxin B. Altogether, the data indicate that C. difficile toxin B acts directly or indirectly on Rho proteins to inhibit ADP-ribosylation and suggest that the cytotoxic effect of toxin B involves Rho.

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DOCUMENT TYPE: Journal
LANGUAGE: English
AB In S. cerevisiae, the **ras**-related protein Rho1p is essentially the only target for ADP-ribosylation by exoenzyme C3 of Clostridium botulinum. Using C3 to detect Rho1p in subcellular fractions, Rho1p was found primarily in the 10,000 g pellet (P2) contg. large organelles; small amts. also were detected in the 100,000 g pellet (P3) and cytosol. When P2 organelles were sepd. in sucrose d. gradients, Rho1p comigrated with the Kex-2 activity, a late Golgi marker. Rho1p distribution was shifted from P2 to P3 in several mutants that accumulate post-Golgi vesicles. Rho1p comigrated with post-Golgi transport vesicles during fractionation of P3 organelles from wild-type or sec6 cells. Vesicles contg. Rho1p were of the same size but different d. than those bearing Sec4p, a **ras**-related protein located both on post-Golgi vesicles and the plasma membrane. Immunofluorescence microscopy detected Rho1p as a punctate pattern, with signal concd. towards the cell periphery and in the bud. Thus, in S. cerevisiae Rho1p resides primarily in the Golgi app. and also in vesicles that are likely to be early post-Golgi vesicles.

L17 ANSWER 21 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 12
ACCESSION NUMBER: 1990:136524 CAPLUS
DOCUMENT NUMBER: 112:136524
TITLE: Multiple small molecular weight guanine nucleotide-binding proteins in human erythrocyte membranes
AUTHOR(S): Damonte, Gianluca; Sdraffa, Adina; Zocchi, Elena; Guida, Lucrezia; Polvani, Carolina; Tonetti, Michela; Benatti, Umberto; **Boquet, Patrice**; De Flora, Antonio
CORPORATE SOURCE: Dep. Biochem., Univ. Genoa, Genoa, 16132, Italy
SOURCE: Biochem. Biophys. Res. Commun. (1990), 166(3), 1398-405
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Native membranes from human erythrocytes contain the following G proteins which are ADP-ribosylated by a no. of bacterial toxins: Gi.alpha. and G0.alpha. (pertussis toxin), Gs.alpha. (cholera toxin), and 3 proteins of 27, 26, and 22 kDa (exoenzyme C3 from Clostridium botulinum). Three addnl. C3 substrates (18.5, 16.5, and 14.5 kDa) appeared in conditions of unrestrained proteolysis during hemolysis. SDS-PAGE sepn. of erythrocyte membrane proteins followed by electroblotting and incubation of nitrocellulose sheets with radiolabeled GTP revealed consistently 4 GTP-binding proteins with Mr values of 27, 26, 22, and 21 kDa. Although a 22-kDa protein was immunochem. identified as **ras** p21, the C3 substrate of 22 kDa is a different protein, probably identifiable with a rho gene

product. Accordingly, at least 5 distinct small-mol.-wt. guanine nucleotide-binding proteins, whose functions are so far undetd., are present in native human erythrocyte membranes.

L17 ANSWER 22 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 13
ACCESSION NUMBER: 1989:419628 CAPLUS
DOCUMENT NUMBER: 111:19628
TITLE: The mammalian G protein rhoC is ADP-ribosylated by Clostridium botulinum exoenzyme C3 and affects actin microfilaments in Vero cells
AUTHOR(S): Chardin, P.; **Boquet, P.**; Madaule, P.; Popoff, M. R.; Rubin, E. J.; Gill, D. M.
CORPORATE SOURCE: Fac. Med. Lariboisiere Saint-Louis, Paris, 75010, Fr.
SOURCE: EMBO J. (1989), 8(4), 1087-92
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English
AB C. botulinum C3 is a recently discovered exo-enzyme that ADP-ribosylates an eukaryotic GTP-binding protein in the **ras** superfamily. Bacterially-expressed product of the human rhoC gene is ADP-ribosylated by C3 and corresponds in size, charge and behavior to the dominant C3 substrate of eukaryotic cells. C3 treatment of Vero cells results in the disappearance of microfilaments and in actinomorphic shape changes without any apparent direct effect upon actin. Thus the ADP-ribosylation of a rho protein seems to be responsible for microfilament disassembly and the unmodified form of a rho protein may be involved in cytoskeletal control.

L17 ANSWER 23 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 14
ACCESSION NUMBER: 1988:108713 CAPLUS
DOCUMENT NUMBER: 108:108713
TITLE: Functional modification of a 21-kilodalton G protein when ADP-ribosylated by exoenzyme C3 of Clostridium botulinum
AUTHOR(S): Rubin, Eric J.; Gill, D. Michael; **Boquet, Patrice**; Popoff, Michel R.
CORPORATE SOURCE: Sch. Med., Tufts Univ., Boston, MA, 02111, USA
SOURCE: Mol. Cell. Biol. (1988), 8(1), 418-26
CODEN: MCEBD4; ISSN: 0270-7306
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Exoenzyme C3 from C. botulinum types C and D specifically ADP-ribosylated a 21-kilodalton cellular protein, p21.bot. Guanyl nucleotides protected the substrate against denaturation, which implies that p21.bot is a G protein. When introduced into the interior of cells, purified exoenzyme C3 ADP-ribosylated intracellular p21.bot and changed its function. NIH 3T3, PC12, and

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COUNTRY OF AUTHOR: USA; JAPAN; FRANCE; ITALY
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (26 APR 1994) Vol. 91, No. 9, pp. 3814-3818.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 45
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cytotoxic necrotizing factor type 2 (CNF2) produced by Escherichia coli strains isolated from intestinal and extraintestinal infections is a dermonecrotic toxin of 110 kDa. We cloned the CNF2 gene from a large plasmid carried by an Escherichia coil strain isolated from a lamb with septicemia. Hydropathy analysis of the deduced amino acid sequence revealed a largely hydrophilic protein with two potential hydrophobic transmembrame domains. The N-terminal half of CNF2 showed striking homology (27 % identity and 80 % conserved residues) to the N-terminal portion of Pasteurella multocida toxin. Methylamine protection experiments and immunofluorescence studies suggested that CNF2 enters the cytosol of the target cell through an acidic compartment and induces the reorganization of actin: into stress fibers. Since the formation of stress fibers in eukaryotic cells involves Rho proteins, we radiolabeled these small GTP-binding proteins from CNF2-treated and control cells with a Rho-specific ADP-ribosyltransferase. The [P-32]ADP-ribosylated Rho proteins from CNF2-treated cells migrated slightly more slowly in SDS/PAGE than did the labeled proteins from the control cells. This shift in mobility of Rho proteins in SDS/PAGE was also observed when CNF2 and the RhoA protein were coexpressed in E. coil. We propose that Rho proteins are the targets of CNF2 in mammalian cells.

L17 ANSWER 20 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 11
ACCESSION NUMBER: 1991:627970 CAPLUS
DOCUMENT NUMBER: 115:227970
TITLE: The small GTP-binding protein Rho1p is localized on the Golgi apparatus and post-Golgi vesicles in Saccharomyces cerevisiae
AUTHOR(S): McCaffrey, Mary; Johnson, Joni S.; Goud, Bruno; Myers, Alan M.; Rossier, Jean; Popoff, Michel R.; Madaule, Pascal; **Boquet, Patrice**
CORPORATE SOURCE: Lab. Physiol. Nerveuse, CNRS, Gif-sur-Yvette, 91198, Fr.
SOURCE: J. Cell Biol. (1991), 115(2), 309-19
CODEN: JCLBA3; ISSN: 0021-9525
Searcher : Shears 308-4994